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(71) Applicant *(for all designated States except US)*: **7TM PHARMA A/S** [DK/DK]; Fremtidsvej 3, DK-2970 Hørsholm (DK).

(71) Applicant *(for US only)*: **SCHWARTZ, Thue, W.** [DK/DK]; Steen Blichers VEj 13, st., DK-2000 Frederiksberg (DK).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **LANGE, Birgitte, Holst** [DK/DK]; Ved Klosteret 2,2.th, DK-2100 Copenhagen Ø (DK).

(74) Agent: **ALBIHNS A/S**; H.C. Andersens Boulevard 49, DK-1553 Copenhagen V (DK).

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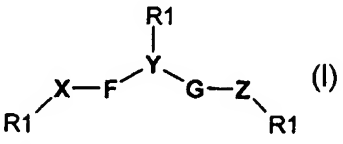
(54) Title: METHOD FOR THE TREATMENT OF MC RECEPTOR RELATED DISORDERS WITH A CHELATE AND/OR A CHELATOR



(I)

(57) Abstract: The present invention relates to a method for reducing overweight and/or treating and/or preventing overweight, obesity and/or complications to obesity (e.g. diabetes type 2, hypertension, hypercholesterolemia, hypertriglyceridemia, cardiovascular diseases and/or orarthritic diseases). The method comprises administering to an animal such as, e.g. a human and/or domestic animal need thereof an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with a natural metal-ion binding site in an MC receptor (melanocortin receptor) from an MC-R family. The invention also relates to methods for treating and/or preventing diabetes mellitus type II ,conditions involving the immune system, inflammation, male or female sexual dysfunctions including erectile dysfunction, anorexia and other appetite disorders, steroidal disorders and perspiration disorders. Furthermore, the invention relates to a cosmetic method for reducing overweight, to the use of a chelate and/or a chelator for the preparation of a pharmaceutical composition and to the use of a chelate and/or chelator as a lead compound in a drug discovery process for identifying ligands that ineract with an MC receptor. The example compounds of the invention are heterocyclic compounds, e.g. bipyridine derivatives.

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METHOD FOR THE TREATMENT OF MC RECEPTOR RELATED DISORDERS WITH A CHELATE AND/OR A CHELATOR

FIELD OF THE INVENTION

The present invention relates to a method for reducing overweight and/or treating and/or preventing overweight, obesity and/or complications to obesity. The method comprises administering to an animal such as, e.g. a human and/or a domestic animal in need thereof an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with a natural metal-ion binding site in an MC receptor from an MC-R family. The invention also relates to methods for treating and/or preventing diabetes mellitus type II, conditions involving the immune system, inflammation, and male or female sexual dysfunctions including erectile dysfunction. Furthermore, the invention relates to a cosmetic method for reducing overweight and to the use of a chelate and/or a chelator for the preparation of a pharmaceutical composition.

BACKGROUND OF THE INVENTION

Obesity is recognized as a major risk factor for coronary heart diseases, hypertension, and type II diabetes mellitus making its treatment and prevention an important health issue. Body weight regulation is a complex process, where multiple environmental and genetic factors contribute to the phenotype. Identification of genes mutated in several animal models of obesity has provided an entry point into understanding the molecular basis of energy homeostasis.

From animal models, Pro-opiomelanocortin (POMC) derived peptides have been recognized to affect food intake and obesity. Several lines of evidence support the notion that the G-protein coupled receptors (GPCRs) of the melanocortin receptor (MC-R) family, are targets of POMC derived peptides involved in the control of for example food intake and metabolism. A specific single MC-R that may be targeted for the control of obesity has not yet been identified, although the MC-3 and MC-4 receptors are the most likely candidates.

The melanocortins, adrenocortcotropic hormone (ACTH), α-, β- and γ-melanocyte-stimulating hormone (MSH), are all derived from the precursor protein pro-opiomelanocortin. They are peptide hormones, which exert their function on the five different members of the melanocortin receptor family. The receptors are widely

distributed both peripherally in the body and in the central nervous system. The physiological functions of the receptors cover a correspondingly diverse spectrum ranging from regulation of pigmentation and immune functions (MC-1 receptor), adrenal cortical steroidogenesis (MC-2/ACTH receptor) and exocrine secretion (MC-5 receptor) to energy homeostasis and food intake (MC-3 and -4 receptors).

As mentioned above, five distinct MC-R's have thus far been identified, and these are expressed in different tissues. MC-1R was initially characterized by dominant gain of function mutations at the Extension locus, affecting coat color by controlling phaeomelanin to eumelanin conversion through control of tyrosinase. MC-1R is mainly expressed in melanocytes and on certain cells of the immune system, such as the monocytes/macrophages, neutrophils, endothelial and mast cells. MC-2R represents the ACTH receptor, responsible for the adrenocortical steroid synthesis and it is expressed in the adrenal gland. MC-3R is expressed in the brain, gut, and placenta and may be involved in the control of food intake, energy homeostasis and thermogenesis. MC-4R is uniquely expressed in the brain and has been shown to be strongly involved in food intake. MC-5R is expressed in many tissues, including white fat, placenta, and exocrine glands. A low level of expression is also observed in the brain. MC-5R knockout mice reveal reduced sebaceous gland lipid production.

One of the first evidences for the involvement of MC-R's in obesity was the discovery that the agouti (ayv) mouse, which ectopically expresses a protein which functions as an antagonist of the MC-1R, MC-3R and-4R, is obese. This indicates that blockade of the action of one or more of these three MC-R's can lead to hyperphagia and metabolic disorders. Furthermore it was found that the MC-4R knockout mice (Huszar et al., Cell, 88,131-141,1997) had the same phenotype as the agouti mice. Importantly, in humans suffering from pathological obesity mutations occur in the MC-4 receptor gene in 1-5% of the cases; which underlines the crusioal importance of the MC-4 receptor in the control of food intake. Importantly, injection intracerebroventricularly (ICV) of the cyclic heptapeptide MT-II (a non-selective MC-1R,-3R,-4R, and-5R agonist) or other MC receptor agonists in rodents, reduces food intake in several animal feeding models (NPY, oblob, agouti, fasted) whereas injection ICV of the selective MC-3R and MC-4R antagonist SHU- 9119 can reverse this effect and induces hyperphagia.

The melanocortin receptors differ from most other 7TM receptors by having a very complex endogenous regulation. In addition to, at least three different peptide agonists (α-MSH, γ-MSH and ACTH) that are acting on the receptors, also endogenous antagonists /

inverse agonists are regulating the function with a high potency. The agonist ligands exhibit certain degree of receptor selectivity: the MC-1 receptor, MC-4 receptor and MC-5 receptor all prefers α-MSH, the MC-3 receptor prefers γ-MSH whereas the MC-2 receptor prefers ACTH. Similar selectivity is observed for the two different antagonists, Agouti and Agouti related peptide (AGRP). The Agouti protein preferentially binds to the MC-1, MC-2 and MC-4 but inhibit MC-2 and MC-5 to a lower extent. The other antagonist, AGRP displays a higher degree of selectivity as it only inhibits MC-3 and MC-4. Due to the constitutive expression of the endogenous antagonist/inverse agonist, even a partial agonist may in the MC receptor system be sufficient to shift the balance in favor of an increased receptor activity. Thus, it is very likely that partial agonist drugs as many of those presented in the present invention will be useful drugs in the treatment of, for example obesity.

Recently the MC-3R knock out phenotype revealed that the MC-3R receptor is involved in regulation of obesity through a different mechanism compared to the MC-4 receptor. The MC-3R KO mice were not or only slightly overweight, but the fat mass of MC-3R KO was approximately the double of that of the wild type, whereas the lean body mass was reduced. On a high fat diet the MC-3R KO mice had a significant higher incidence of obesity compared to wild type mice on the same diet. In contrast to the MC-4R KO mice these mice did not exhibit increased food intake – they even were hypophagic in some groups. Furthermore the MC-3R KO mice had a lower locomotor activity, which were statistically significant compared to wild type mice. The MC-3 receptor is believed to be expressed for example on the NPY / AGRP neurons in the arcuate nucleus and here be part of the system where the MSH from the POMC expressing neurons inhibits the stimulatory branch of the appetite control also in the arcuate nucleus while it acts through MC-4 receptors in the paraventricular nucleus to serve the same purpose of inhibiting food intake.

Synthetic melanocortin receptor agonists (melanotropic peptides) have been found to initiate erections in men with psychogenic erectile dysfunction. Activation of melanocortin receptors of the brain appears to cause normal stimulation of sexual arousal. It has been demonstrated that a centrally acting α-melanocyte-stimulating hormone analog, melanotan-II (MT-II), exhibited a 75% response rate, similar to results obtained with apomorphine, when injected intramuscularly or subcutaneously to males with psychogenic erectile dysfunction. Some studies indicate that the effect of MC receptor agonists on penile erection may also occur locally in the penis.

DISCLOSURE OF THE INVENTION

During the last decade research has been carried out in order to find suitable substances for use in the treatment of obesity. As discussed above, focus has mainly been on the MC receptor family and the development of peptide based substances. Although recently the first non-peptide MC receptor agonists have also been described.

The present inventors have found that a specific class of chemical substances acts as agonists both in a MC-1 and a MC-4 receptor model. The common feature of these substances is their ability to act as chelators, i.e. there ability to form a complex with a metal ion. These results have led the inventors to investigate whether the MC receptors contain a metal-ion binding site and the present invention is based on this finding, i.e. that MC receptors contain a natural metal-ion binding site, which can be used as a target for the treatment of e.g. obesity, erectile dysfunctions, and inflammation.

Metal ions have been applied to stabilise agonists for the MC receptor family, but there have been no suggestions or indications of any interaction between a metal ion and the receptor itself.

Naturally occurring metal-ion binding sites in MC receptors can be used as targets or attachment sites for metal-ion chelating compounds. In general, such natural metal-ion sites are identified functionally by studying the effect of either free metal-ions and/or by the effect of metal-ion chelators or chelates on any function of the receptor. Metal-ion binding sites can also be identified or confirmed by structural means and location of the site can also be identified by careful, controlled mutagenesis, i.e. exchanging of the residues involved in metal-ion binding with residues not having this property. The metal-ion site involved in the action of the metal-ion and the metal-ion chelating compounds has in this way been localized to residues in the MC-1 receptor, which are conserved among all the MC receptors.

The present inventors have found that Zn^{2+} modulates the activity of both MC-1 receptor and MC-4 receptor. In both receptors the affinity for zinc ions were approximately 15 μM as measured in competition binding studies. Furthermore, functional studies reveal that the zinc ion binding induces an active conformation of the receptor. Not only zinc ions alone but also zinc ions chelated with metal ion chelators are able to activate both of the two receptors tested. Thus, metal ions potentially represent a novel starting point in the drug discovery for melanocortin receptor agonists. An additional advantage of the metal

ion as a drug discovery lead is that it has potential as an enhancer of the α-MSH induced activation. In the case of the MC-1 receptor a six-fold leftwards shift in the dose-response curve for the endogenous MSH hormone was observed. Such a positive modulation of the function of the normal hormonal control of the MC receptors is a novel and potential highly beneficial way of shifting the activity towards higher activity. It is contemplated that the metal ion induced modulation of the endogenous melanocortin receptor activity has physiological relevance.

Definitions

Throughout the text including the claims, the following terms shall be defined as indicated below.

"*Agouti*" is intended to indicate an endogenous protein which acts as an antagonist or inverse agonist at the MC-1, MC-2, MC-4 and to a lower extent also the MC-5 receptors

"*AGRP*" is intended to indicate the agouti related proyein, which is homologous to agouti and acts as an endogenous antagonist or inverse agonist preferentially at the MC-3 and MC-4 receptors.

In the present context the term *"body mass index"* or *"BMI"* is defined as body weight (kg)/height^2 (m^2).

"Overweight" is intended to indicate a BMI in a range from about 25 to about 29.9.

"Obesity" is intended to indicate a BMI, which is at least about 30.

"Erectile dysfunction" is intended to indicate a disorder involving the failure of a male mammal to achieve erection, ejaculation, or both. Symptoms of erectile dysfunction include an inability to achieve or maintain an erection, ejaculatory failure, premature ejaculation, or inability to achieve an orgasm. An increase in erectile dysfunction is often associated with age and although it is generally caused by a physical disease or as a side effect of drug treatment it may also be of psychological nature.

A *"chemical compound"* is intended to indicate a small organic molecule of low molecular weight or a small organic compound, which is capable of interacting with a receptor, in particular with a protein, in such a way as to modify the biological activity thereof. The

term includes in its meaning metal-ion chelates of the formulas shown below. Furthermore, the term includes in its meaning metal-ion chelates of the formulas shown below as well as chemical derivatives thereof constructed to interact with other part(s) of the receptor than the metal-ion binding site. A chemical compound may also be an organic compound, which in its structure includes a metal atom via a covalent binding.

A *"metal-ion chelator"* or a *"chelator"* is intended to indicate a chemical compound capable of forming a complex with a metal atom or ion, and contains at least two interactions between the metal center and the chelator. Such a compound will generally contain two heteroatoms such as N, O, S, Se or P with which the metal atom or ion is capable of forming a complex. A *"metal-ion chelate"* or a *"chelate"* is intended to indicate a complex of a metal ion chelator and a metal atom or ion.

A *"ligand"* is intended to indicate a functional group or a structural element that binds or coordinates a metal ion.

A *"metal ion"* is intended to indicate a charged or neutral element. Such elements belong to the groups denoted main group metals, light metals, transition metals, semi-metals or lanthanides (according to the periodic system). The term "metal ion" includes in its meaning metal atoms as well as metal ions.

A *"metal-ion binding site"* is intended to indicate a part of a receptor that comprises atoms in relative positions in such a way that they are capable of complexing with a metal atom or ion. Such atoms will typically be heteroatoms, in particular N, O, S, Se or P. With respect to proteins a metal-ion binding site is typically an amino acid residue of a protein, which comprises an atom capable of forming a complex with a metal ion. These amino acid residues are typically, but not restricted to, histidine, cysteine, glutamate and aspartate.

A *"receptor-ligand"* is intended to include any substance that binds to a receptor and thereby inhibiting or stimulating its activity. An "agonist" is defined as a ligand increasing the functional activity of a receptor (e.g. signal transduction through a receptor). An "antagonist" is defined as a ligand decreasing the functional activity of a receptor either by inhibiting the action of an agonist or by its own intrinsic activity. An "inverse agonist" (also termed "negative antagonist") is defined as a ligand decreasing the basal functional activity of a receptor.

The term *"endogenous"*, e.g. in the sense "endogenous metal-ion site", is intended to mean that the metal-ion site occurs in the natural unmutated receptor

A *"functional group"* is intended to indicate any chemical entity which is a component part of the chemical compound and which is capable of interacting with an amino acid residue or a side chain of an amino acid residue of the receptor. A functional group is also intended to indicate any chemical entity, which is a component part of the receptor and which is capable of interacting with other parts of the receptor or with a part of the chemical compound. Functional groups may be involved in interactions such as, e.g., ionic interactions, ion-dipole interactions, dipole-dipole interactions, hydrogen bond interactions, hydrophobic interactions, pi-stacking interactions, edge-on aromatic interactions, dispersion and induction forces or metal complex interactions.

The term *"in the vicinity of"* is intended to include an amino acid residue or any other residue or functional group located in the space defined by the binding site of the metal ion chelate and at such a distance from the metal ion binding amino acid residue that it is possible, by attaching suitable functional groups to the chemical compound, to generate an interaction between said functional group or groups and said amino acid residue, another residue or functional group.

Aspects of the Invention

Based on the results described in the Examples herein it is contemplated that chelates and chelators of Formula I below are effective as melanocortin receptor agonists, i.e. agonist for the MC-R series such as, e.g. MC-1R, MC-2R, MC-3R, MC-4R and MC-5R and in particular for MC-1R and/or MC-4R. Especially, they are believed to be useful for the treatment and/or prevention of disorders responsive to the activation of human MC-4R, such as overweight, obesity, diabetes as well as male and/or female sexual dysfunction, in particular, erectile dysfunction, and further in particular, male erectile dysfunction.

The present invention relates to a method for reducing overweight and/or for treating of and/or preventing overweight, obesity and/or complications thereto, the method comprising administering to an animal such as, e.g. a human and/or a domestic animal in need thereof an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with a natural metal-ion binding site in an MC receptor. The complications to overweight and/or obesity may be diabetes type II, hypertension,

hypercholesterolaemia, hypertriglyceridaemia, cardiovascular diseases and/or arthritic diseases.

In an aspect, the invention relates to a method treating and/or prevention diabetes mellitus type II, the method comprising administering to an animal such as, e.g. a human and/or a domestic animal in need thereof an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with a natural metal-ion binding site in an MC receptor.

Furthermore, the invention relates to a cosmetic method for reducing overweight, the method comprising administering to an animal such as, e.g. a human and/or a domestic animal in need thereof an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with a natural metal-ion binding site in an MC receptor.

In a further embodiment the invention relates to a method for reducing the fat tissue mass /lean mass body mass ratio in a domestic animal, the method comprising administering to a domestic animal an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with a natural metal-ion binding site in an MC receptor.

The invention also relates to a method for treating and/or preventing conditions involving the immune system, the method comprising administering to a human or a domestic animal an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor.

Moreover, the invention relates to a method for treating and/or preventing male or female sexual dysfunction such as, e.g. psychogenic sexual dysfunction of a mammal including a human, the method comprising administering to the mammal in need thereof an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor.

Furthermore, the invention relates to a method for treating and/or preventing erectile dysfunction in a mammal including a human, the method comprising administering to the mammal in need thereof an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor.

In all aspects the MC receptor may be selected from the group consisting of MC-1, MC-2, MC-3, MC-4, MC-5 such as, e.g., MC-1 or MC-4, including homo- and heterodimers, -trimers and -oligomers thereof.

In general, the MC receptor mentioned above is a mammalian MC receptor such as, e.g. a human MC receptor, a dog MC receptor, a cat MC receptor, a mouse MC receptor or a rat MC receptor.

The invention also relates to a method for treating and/or preventing conditions involving the immune system, the method comprising administering to an animal such as, e.g. a human in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC-1receptor.

Furthermore, the invention relates to a method for treating and/or preventing chronic and acute inflammation, the method comprising administering to an animal such as, e.g. a human in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC-1receptor. The acute inflammation may be related to ischemic conditions, such as, e.g. ischemic stroke.

In those cases where the chelate and/or chelator have agonistic and/or antagonistic activity against an MC-1 receptor, they can be used in a cosmetic method for obtaining a suitable tan of the skin of an animal including a human. Such a method comprises administering to an animal such as, e.g. a human in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC-1receptor.

The invention also relates to a method for treating and/or preventing anorexia and/or other appetite disorders, the method comprising administering to an animal such as, e.g. a human and/or a domestic animal in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor. In such cases, the chelate and/or chelator must have antagonistic activity against an MC receptor such as an MC-3 and/or MC-4 receptor.

When the chelate and/or chelator have agonistic and/or antagonistic activity against an MC-2 receptor, they can be used in a method for treating and/or preventing steroidal

disorders such as, e.g. Cushing's syndrome. The method comprises administering to an animal such as, e.g. a human in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC-2 receptor.

The invention also relates to a method for treating and/or preventing perspiration disorders such as, e.g. sweat deficiency e.g. hypohidrosis, or excessive sweating e.g. diaphoresis or hyperhidrosis, the method comprising administering to an animal such as, e.g. a human in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC-5R of an MC-R family. Suitable chelates and/or chelators are those with agonistic and/or antagonistic activity against an MC-5 receptor.

Chelates and chelators for use according to the invention

Besides the chemical structure, the test compounds normally fulfill certain criteria with respect to molecular weight (at the most 3000 such as, e.g., at the most 2000, at the most 1500, at the most 1000, at the most 750, at the most 500), number of hydrogen bond donors (at the most 15 such as, e.g. at the most 13, 12, 11, 10, 8, 7, 6 or at the most 5) and number of hydrogen bond acceptors (at the most 15 such as, e.g. at the most 13, 12, 11, 10, 8, 7, 6 or at the most 5). However, there may be cases where the molecular weight, number of hydrogen bond donors and/or number of hydrogen bond acceptors of a test compound of a library of the invention have other values than the above-mentioned.

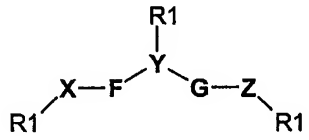
Chemical compounds, which are suitable for use in drug discovery processes involving receptors having a metal-ion site, are any compounds that are capable of forming a complex with a metal ion.

More specifically, a chemical compound for use according to the invention has at least two heteroatoms, similar or different, selected from the group consisting of nitrogen (N), oxygen (O), sulphur (S), selenium (Se) and phosphorous (P).

Chemical compounds, which have been found to be useful in the present invention, are typically compounds comprising a heteroalkyl, heteroalkenyl, heteroalkynyl moiety or a heterocyclyl moiety for chelating the metal ion.

The term "heteroalkyl" is understood to indicate a branched or straight-chain chemical entity of 1-15 carbon atoms containing at least one heteroatom. The term "heteroalkenyl" is intended to indicate a branched or straight-chain chemical entity of 2-15 carbon atoms containing at least one double bond and at least one heteroatom. The term "heteroalkynyl" is intended to indicate a branched or straight-chain chemical entity of 2-15 carbon atoms containing at least one triple bond and at least one heteroatom. The term "heterocyclyl" is intended to indicate a cyclic unsaturated (heteroalkenyl), aromatic ("heteroaryl") or saturated ("heterocycloalkyl") group comprising at least one heteroatom. Preferred "heterocyclyl" groups comprise 5- or 6-membered rings with 1-4 heteroatoms or fused 5- or 6-membered rings comprising 1-4 heteroatoms. The heteroatom is typically N, O, S, Se or P, normally N, O or S. The heteroatom is either an integrated part of the cyclic, branched or straight-chain chemical entity or it may be present as a substituent on the chemical entity such as, e.g., a thiophenol, phenol, hydroxyl, thiol, amine, carboxy, etc. Examples of heteroaryl groups are indolyl, dihydroindolyl, furanyl, benzofuranyl, pyridyl, pyrimidyl, pyrazoyl, benzothiazoyl, quinolinyl, triazolyl, imidazolyl, thiazolyl, tetrazolyl and benzimidazolyl. The heterocyclyl group generally includes 2-20 carbon atoms, and 1-4 heteroatoms.

Particularly interesting chemical compounds to use according to the present invention are those having at least two heteroatoms connected according to the general formula I abbreviated as Che-R1



Formula I

wherein F is N, O, S, Se or P; and G is N, O, S, Se or P;

X, Y and Z, which are the same or different, are straight or branched C_1-C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} alkynyl, C_1-C_{12} cyclyl, aryl, C_1-C_{12} heteroalkyl, C_1-C_{12} heteroalkenyl, C_1-C_{12} heteroalkynyl, C_1-C_{12} heterocyclyl, heteroaryl;

R1 may be present anywhere on the X, Y and/or Z moiety and it may be present on X, Y and/or Z up to as many times as possible, i.e. if X is –CH2-CH2-, then R1 may be present

on the first and/or second carbon atom one or several times; R1 could optionally be hydrogen;

X may together with Y and/or Z fuse to form a cyclic ring system;
Y may together with X and/or Z fuse to form a cyclic ring system;
X, Y and Z may together fuse to form a cyclic ring system;

R^1 corresponds to a structure –A-B-C, wherein the element A is a coupling or connecting moiety, B is a spacer moiety and C is a functional group; –B- may be substituted one or more times with a further C, which may be the same or different, and

A linked to be –A-B-C is selected from the group consisting of:

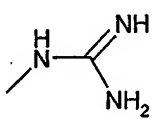
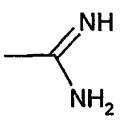
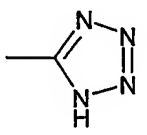
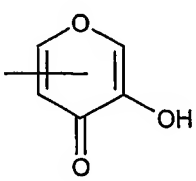
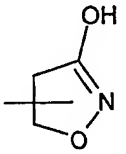
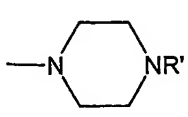
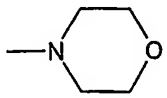
-O-, -S-, -NH-, -N=, -N<, -CH_2-, -C(=O)-, -PO_3-, -PO_2NH-, -$NHPO_2$, -NHP(O)<, -C≡C-, -CH=CH-, -SO-, -SO_2-, -COO-, -CONR"-, -NR'CO-, -NR'SO_2-, -SO_2NR"-, -CH(OH)-, -CR'(OH)-, -CR'(O-alk)-, -N-alk-, aryl, cycloalkyl, heteroaryl, heterocycloalkyl etc., and the term "alk" includes straight or branched alkyl, straight or branched alkenyl and straight or branched alkynyl; R' is H or lower alk, i.e. C_1-C_6; R" is as defined below;

-B- is absent or selected from the group consisting of:

H, alkyl, straight or branched alkyl, alkenyl (straight or branched), alkylnyl (straight or branched), aryl, cycloalkyl, heteroaryl, heterocycloalkyl, alkyloxyalkyl, alkylaminoalkyl,

-C is absent or selected from the group consisting of:

-H, -OH, -NR"R"', -CONR"R"', -COOR", -OCOR", -COR", -SO_2NR"R"', -SH, -S-S-alk, -NHCOR", -NR"COR"', $NHSO_2$R", -$NHCONH_2$, -NH-CN, -F, -Cl, -Br, -I; -SCF_3, -CF_3, -OCF_3, -SCH_3, -SR", -CN, -$N(CN)_2$,-NO_2, -OCH_3, -OR', -NH_2, -NHAlk, -NHMe, -$NHAlk_2$, -NMe_2, -NMeAlk, -$N(Alk)_3^+$, heteroaryl, heterocycloalkyl

and R'' and/or R''' has the same meaning as given for B above optionally substituted with one or more C;

in those cases where a compound has two or more R^1 in positions adjacent to each other the –A- and/or –B- elements from the two individual R^1 may form a cyclic ring system;

in those cases where B is absent R^1 is –A-C or –A and in those cases where C is absent R^1 is –A-B or –A;

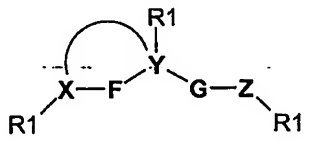
in some cases, A may be absent and then $-R^1$ is –B-C or –C, and B may be substituted one or more times with C, which may be the same or different;

the total number of atoms (X+F+Y+G+Z) excluding hydrogen atoms is at the most 25;

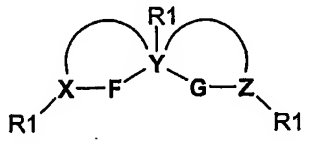
the total number of heteroatoms in (X+F+Y+G+Z) is at the most 6; and

the size of a ring is at the most 14 atoms, preferably 5 or 6 atoms.

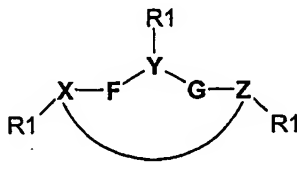
As mentioned above X, Y and/or Z may fuse to form one or more rings. Thus, X-F-Y may be part of a heterocyclyl ring system:



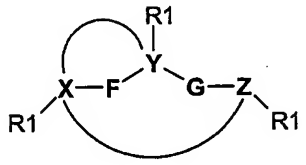
Alternatively, X-F-Y and Y-G-Z may be part of heterocyclyl ring systems:



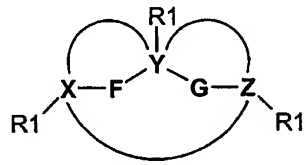
X-F-Y-G-Z may also be part of heterocyclyl ring systems:



X-F-Y and X-F-Y-G-Z may be part of heterocyclyl ring systems:



Furthermore, X-F-Y and Y-G-Z and X-F-Y-G-Z may be part of heterocyclyl ring systems:



In the present context, the term "alkyl" is intended to indicate a branched or straight-chain, saturated chemical group containing 1-15 such as, e.g. 1–12, 1-10, preferably 1-8, in particular 1-6 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, sec. butyl, tert. butyl, pentyl, isopentyl, hexyl, isohexyl, heptyl etc.

The term "alkenyl" is intended to indicate an unsaturated alkyl group having one or more double bonds.

The term "alkynyl" is intended to indicate an unsaturated alkyl group having one or more triple bonds.

The term "cycloalkyl" is intended to denote a cyclic, saturated alkyl group of 3-7 carbon atoms.

The term "cycloalkenyl" is intended to denote a cyclic, unsaturated alkyl group of 5-7 carbon atoms having one or more double bonds.

The term "aryl" is intended to denote an aromatic (unsaturated), typically 6-membered, ring, which may be a single ring (e.g. phenyl) or fused with other 5- or 6-membered rings (e.g. naphthyl or anthracyl).

The term "alkoxy" is intended to indicate the group alkyl-O-.

The term "amino" is intended to indicate the group –NR''R''' where R'' and R''' which are the same or different, have the same meaning as R'' in formula I. In a primary amine group, both R'' and R''' are hydrogen, whereas in a secondary amino group, either but not both R'' and R''' is hydrogen. In a tertiary amino group neither of R'' and R''' is hydrogen. R'' and R''' may also be fused to form a ring.

The term "ester" is intended to indicate the group COO-R'', where R'' is as indicated above except hydrogen, -OCOR'', or a sulfonic acid ester or a phosphonic acid ester.

In the formula I above it is contemplated that if the valency of the heteroatoms F and/or G is more than 2 then further X, Y and/or Z groups may be present adjacent to the F and/or G groups.

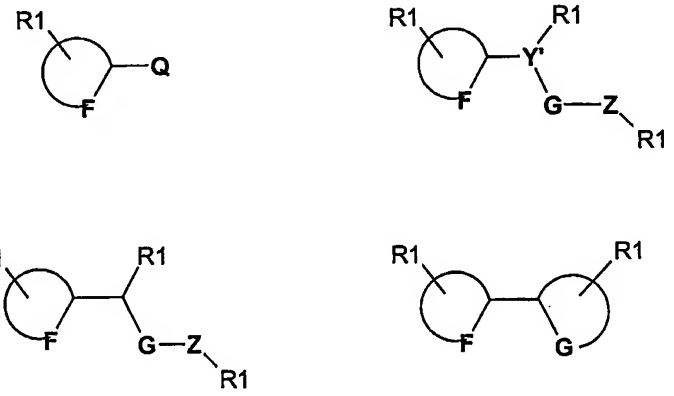
Specific structures

In one embodiment, the invention relates to the use of chemical compounds having a specific characteristic feature in common.

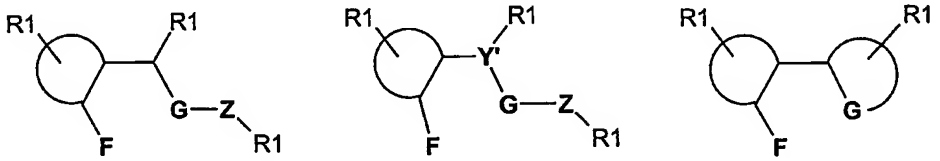
Chemical compounds of the following general formulas are of specific interest in the present context. The following formulas are based on the formula I above and F and/or G have the same meaning as indicated above, i.e. F and/or G are heteroatoms. Q is a structural element containing a heteroatom. A circle indicates a cyclic alkyl, alkenyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl or heteroaryl ring having from 3-7 atoms in the ring. R^1 has the same meaning as indicated above and, when more than one R^1 is present they may be the same or different. If no specific position is given for the radical, the radical may be placed anywhere in the cyclic system and there may also be as many radicals as there is positions possible in the structure. Other symbols employed in the formulas below have the same meaning as given under formula I above. In the formulas below, the structure of the compounds are given in different structure levels. First in a very general form and then in more and more specific forms.

More specifically, compounds of interest have one of the following structures. Y' is the remainder of the group Y which also includes Y'' being absent, i.e. G being directly linked to the ring. The coordinating atom F is included in a 5- or 6-membered aromatic,

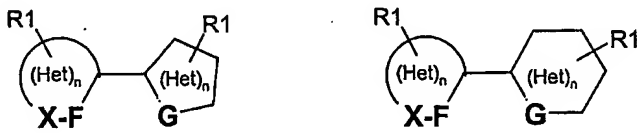
unsaturated or saturated heterocycle containing between one and three heteroatoms and the coordinating atom G is either included in a 5- or 6-membered aromatic, unsaturated or saturated ring or an open chain. Preferably, F is N, O or S; and G is N, O or S:



In the following subclass the coordinating atom F is appended to an aromatic, unsaturated or saturated 5- or 6-memebered ring. Preferably, F is N, O or S; and G is N, O or S.

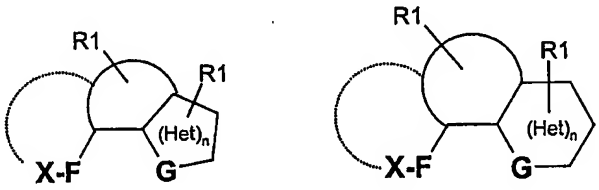


In the following biheterocyclyl subclass the coordinating atom G is included in a 5-or 6-membered aromatic, unsaturated or saturated heterocycle containing between one and four heteroatoms and the coordinating atom F contained within an aromatic, unsaturated or saturated 5- or 6-memebered heterocycle containing between one and four heteroatoms. Preferably, F is N, O or S; and G is N, O or S.

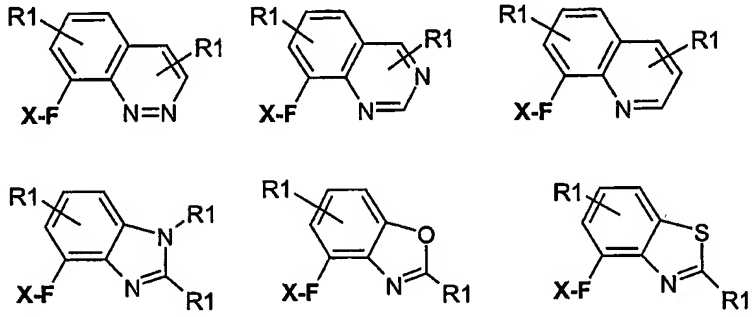


In the following subclass the coordinating atom G is included in a 5- or 6-membered aromatic, unsaturated or saturated heterocycle containing between one and three

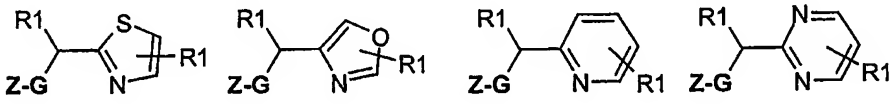
heteroatoms and the coordinating atom F appended to an annelated aromatic, unsaturated or saturated 5- or 6-memebered ring. X-F can optionally be included in a fused ring as indicated by the dashed line. Preferably, F is N, O or S; and G is N, O or S.



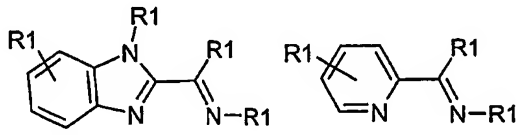
The annelated derivatives may be substituted with one or more R^1 moieties. Thus, a compound for use according to the present invention may be mono-, di-, tri-, tetra-, pentasubstituted derivatives.



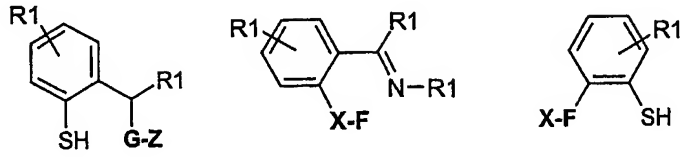
Suitable heterocyclic coordinating rings could be appended with coordinating moieties G to produce other chelating scaffolds containing one or more R1 groups.



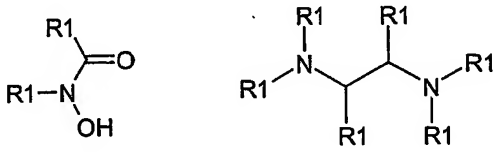
Typical coordinating sacffolds of this type are imine moieties appended to coordinating heterocycles.



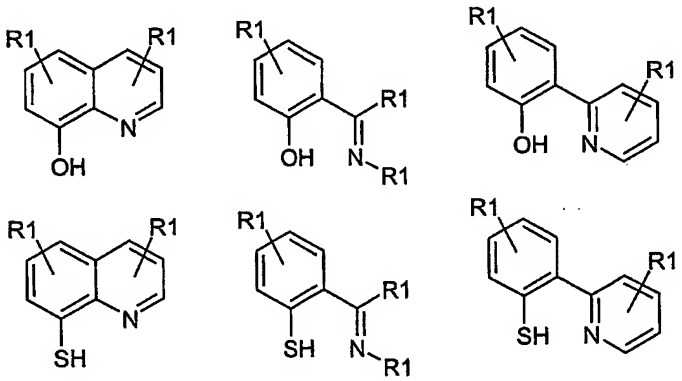
Alternatively, the coordinating groups, e.g. thiol and imine, may be attached to a ring moiety containing one or more R1 groups.



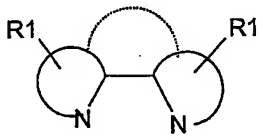
Other suitable open-chain chelating scaffolds are hydroxamic acids or 1,2-diamine coordinating moieties containing one or more R1 groups.



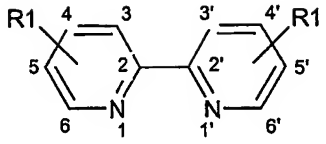
Chelator scaffolds containing one or more R1 groups of particular value are:



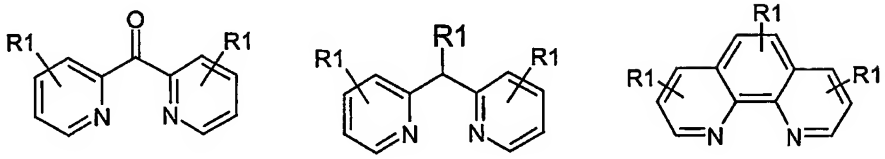
Useful nitrogen containing biheterocyclyl chelator scaffolds of particular interest are:



and, especially, pyridine containing systems of the following type



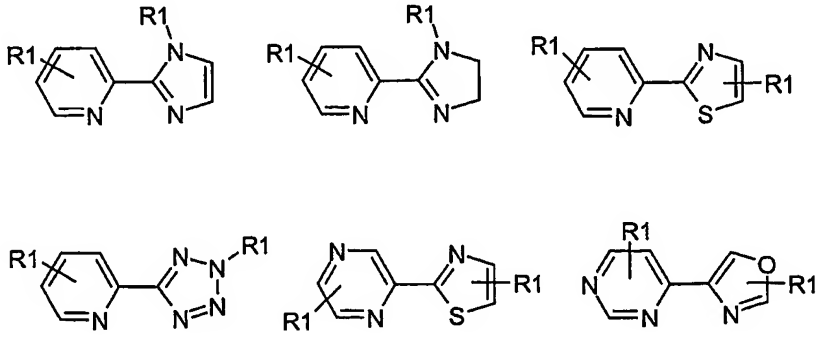
Other useful pyridyl-containg systems are systems such as



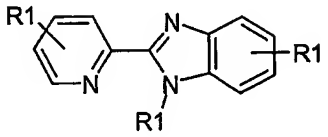
2-pyridyl systems may also be connected to other six-membered nitrogen containing rings having one nitrogen adjacent to the connecting bond, such as

Non-pyridyl six-membered nitrogen containing aromatic rings may also be coupled to another non-pyridyl six-membered nitrogen containing ring where both ring systems having one nitrogen adjacent to the connecting bond, form useful scaffolds

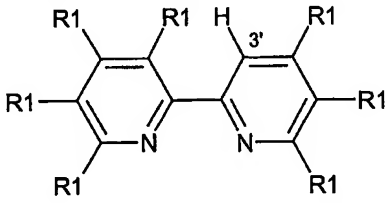
The following biheterocyclyl derivatives may be substituted with one or more R^1 moieties. Thus, a compound for use according to the present invention may be mono-, di-, tri-, tetra-, pentasubstituted biheterocyclyl derivatives. The biheterocyclyl system may be symmetric or asymmetric and they may be symmetricly or asymmetricly substituted with one or more R1 groups.



The 5-membered ring may also be annelated with e.g. a benzene ring.

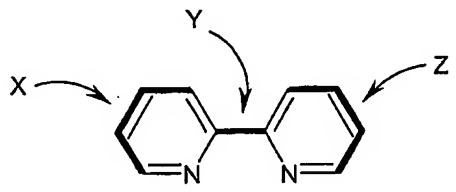


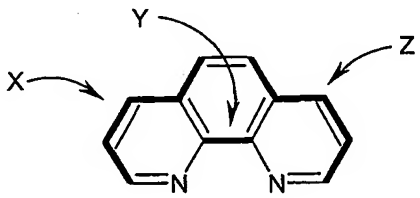
In the figure below, 2,2'-bipyridine is given as an example on a common basic structural element for chemical compounds for use according to the invention, i.e. the 2,2'-bipyridine here functions as the chelator skeleton.

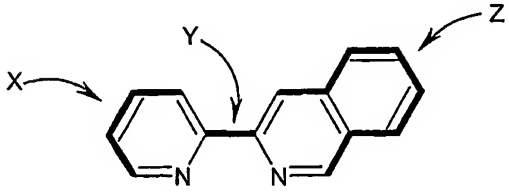


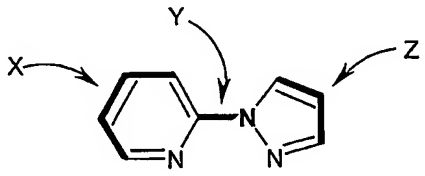
The chemical exemplifications and functionalisation principles given on this skeleton can be applied in analogous manner for other scaffolds with proper adjustments for adoption of suitable chemical routes for the different chelator systems, i.e. Che-R^1 or more specifically Che-A-B-C, wherein Che constitutes the different chelating scaffolds derived from Formula I and described above optionally substituted further with one or more, the same or different, R^1 or more specifically A-B-C groups.

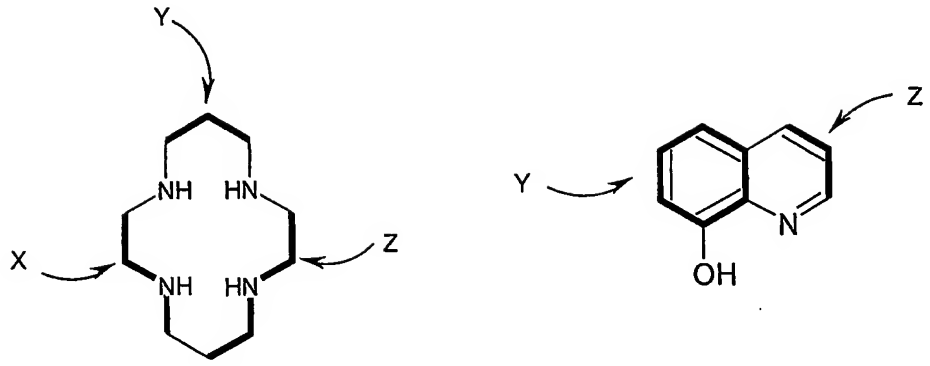
In the following are given some specific structures in which the various elements X, Y and Z are marked with bold.



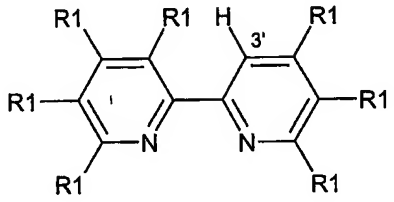








Thus, a suitable compounds for use according to the present invention may be a 2,2'-bipyridine.



Formula II

The construction of compounds for use according to this invention will be exemplified by the use of 2,2´-bipyridines with no intention to exclude other chelating scaffolds including the general Che-R^1 / Che-A-B-C, wherein Che constitutes the different chelating scaffolds optionally substituted further with one or more, the same or different, R^1 or more specifically A-B-C groups.

Accordingly, the 2,2'-bipyridines for use according to the invention is normally substituted with one or more functional groups. Thus, a compound for use according to the present invention may contain mono-, di-, tri-, tetra-, penta-, hexa- or heptasubstituted bipyridines. The di-, tetra- and/or hexasubstituted bipyridines may be symmetric or asymmetric substituted bipyridines. Normally, up to 4 or at the most 5 substituents are present on the 2,2'-bipyridine skeleton. As seen from the formula II above, the position 3' is preferably substituted with a hydrogen atom.

Chemical compounds of the following general formulas are of specific interest in the present context. The groups of compounds are denoted

i) "A-group" in those cases where the compounds have a common connecting element, -A-, and

ii) "C-group" in those cases where the compounds have a common functional group, –C.

In those cases where the 2,2'-bipyridines are disubstituted, the group of compounds may be an AA-, AA'-, AC-, CC- or CC'-group (A' is different from A but selected from the same group as A mentioned above; the same applies to C and C').

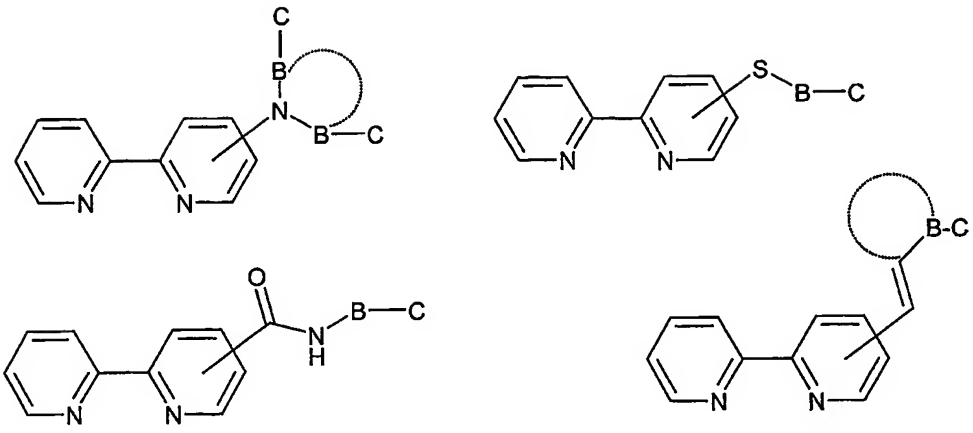
For trisubstituted 2,2'-bipyridine, the group of compounds may be an AAA-, AAA'-, AA'A"-, AAC-, AA'C-, ACC-, ACC'-, AC'C"-, CCC-, CCC'- or CC'C"- group (or other possible permutations; the same notation is used as above, i.e. A is different from A', and A and A' are different from A"). The same notation applies for tetra-, penta-, hexa- or heptasubstitued 2,2-bipyridines.

A-group

The 2,2'-bipyridines of an A-group have a common connecting group attached directly on the ring system and a variable B-C moiety. Examples are compounds according to formula III above in which A is e.g. –O-, –NH-, -S-, -N=, -N<, -CONH-, -CON<, -COO-, -CH=CH-. The functionalisations are made according to well-known chemical reactions with proper considerations of chemical compatibility of the functional groups with respect to the synthetic steps. Some exemplifications will be shown in the following.

Representative examples are

Che-N(B-C)$_2$; Che-S-B-C ; Che-CO-NH-B-C ; Che-CH=CH-B-C ; Che-O-B-C ; Che-NH-CO-B-C ; Che-SO_2-NH-B-C as exemplified with the Che being 2,2'-bipyridine :

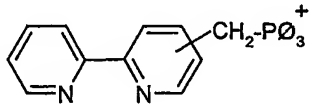


Examples of compounds having an amide -CONH-B-C, an alkene -CH=CH-B-C and a retroamide–NHCO-B-C are detailed in the Experimental part. Note, the B moiety may optionally be part of a ring appended to an exo-cyclic double bond.

The compounds having an amide –CONH-B-C can be obtained by reacting a suitably activated carboxylate derivative with appropriate amines as detailed in the Experimental part. The amines can be obtained by reacting the bipyridyl amines with suitable B-C reagents or sequentially by reaction with a B reagent followed by a C reagent. The alkenes can be obtained by forming the double bond in either direction, i.e. either having the carbonyl moiety on the bipyridyl scaffold or preferably having the carbonyl moiety located on the B moiety as indicated in the example. The thiols may be obtained by alkylation of the thiol with a B-C reagent or by nucleophilic addition/elimination with a suitable sulphur-containing derivative.

In the formula above, the substituent (e.g. –CONH-B-C) may be positioned anywhere in the 3, 4 or 5 position on the 2,2'-bipyridine skeleton.

Alkenes with different B and C moieties can be obtained by reacting ylides of phosphonium salts or phosphonates such as:



wherein Ø means a phenyl group, with appropriate ketone or aldehyde derivatives as detailed in the Experimental part.

AA'groups

Suitable examples are

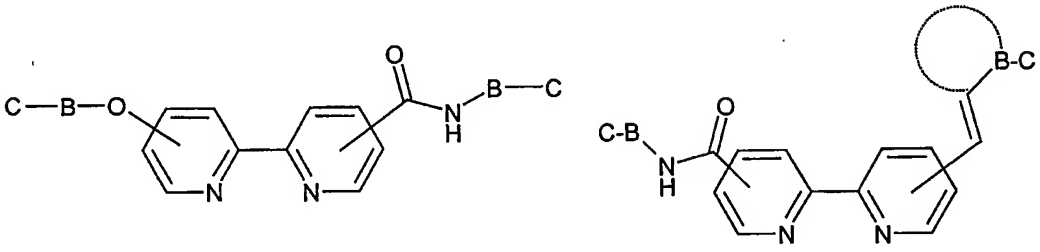
C-B-O-Che-CO-NH-B-C ;

C-B-NH-CO-Che-CH=CH-B-C ;

C-B-NH-CO-Che-NH-CO-B-C ;

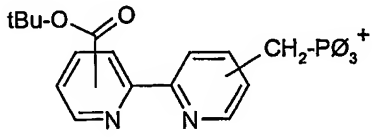
C-B-NH-CO-Che-CO-NH-B-C

as exemplified with Che being 2,2'-bipyridine:



i.e. a disubstituted 2,2'-bipyridines (one ring substituted with a functionalised amide and the other ring substituted with a functionalised ether, –O-B-C or in the other example a functionalised alkene -CH=CH-B-C). Note in the latter case, the B moiety may optionally be part of a ring appended to an exo-cyclic double bond.

Alkenes/amides (AA') with different B and C moieties can be obtained by reacting ylides of phosphonium salts or phosphonates containing suitably protected carboxylic functions such as:

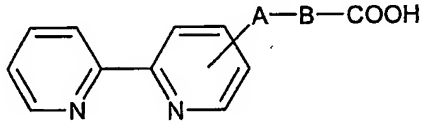


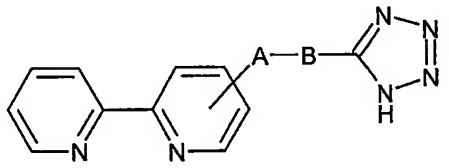
wherein Ø means a phenyl group, with appropriate ketone or aldehyde derivatives, followed by deprotection, activation and coupling with suitable amines as shown for the amides (see Experimental section).

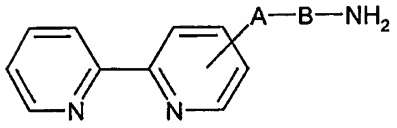
C-group

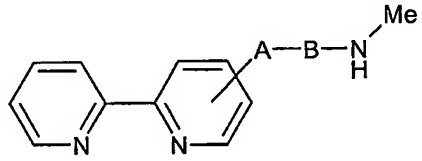
The 2,2'-bipyridines of a C-group have a common functional group either directly attached on the 2,2'-biyridine skeleton or at a position at a distance from the skeleton. Irrespective its position, a characteristic feature of a C-group is that the common functional group is not further derivatized or substituted. Examples are 2,2'-bipyridines of formula II wherein R^1 is –A-B-C, -A-C, -B-C or –C (and, if present, B may be further substituted with one or more C groups). Examples on such functional end groups are e.g. –CHO, $-NH_2$, $-NHCH_3$, -guanidin, -tetrazol, -COOH, -COONa, $-CONH_2$, $-NO_2$, -CN, i.e. Che-A-B–CHO, Che-A-B-NH_2, Che-A-B-$NHCH_3$, Che-A-B-guanidin, Che-A-B-tetrazol, Che-A-B-COOH, Che-A-B-COONa, Che-A-B-$CONH_2$, Che-A-B-NO_2, Che-A-B-CN.

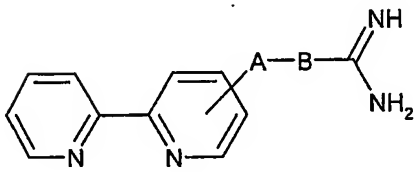
Accordingly, the following formulas represent compounds exemplified with Che being 2,2'-bipyridine:

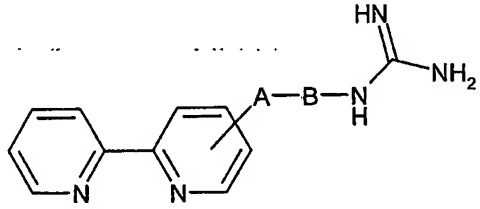












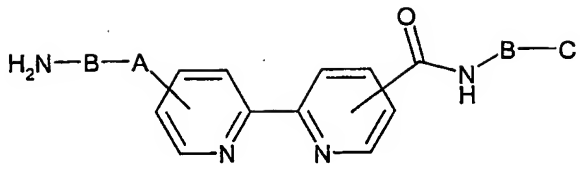
AC-groups

Examples are

Me_2N-B-A-Che-NH-CO-B-C;

HOOC-B-A-Che-CO-NH-B-C

as exemplified by Che being 2,2'-bipyridine:



Some of the chemical compounds of the above-mentioned formulas are already known and are commercially available or may be prepared according to methods known by a person skilled in the art. However, those chemical compounds that are novel are subject to specific embodiments of the present invention and they may be prepared by the following method:

The present invention also relates to symmetric disustituted bipyridines, i.e. the bipyridine skeleton has been substituted in the same position in the two pyridine rings. The substituent may be the same or different and it may represent the same or different functional group.

Metal ions forming the complex with the heteroalkyl or heterocyclyl moiety in the chemical compounds may advantageously be selected from metal ions, which have been tested for or are used for pharmaceutical purposes.
Such metal ions belong to the groups denoted light metals, transition metals, post-transition metals or semi-metals (according to the periodic system).

The metal ion is typically selected from the group consisting of aluminium, antimony, arsenic, astatine, barium, beryllium, bismuth, boron, cadmium, calcium, cerium, caesium, chromium, cobalt, copper, dysprosium, erbium, europium, gadolinium, gallium, germanium, gold, hafnium, holmium, indium, iridium, iron, lanthanum, lead, lutetium, magnesium, manganese, mercury, molybdenum, neodymium, nickel, niobium, osmium, palladium, platinum, polonium, praseodymium, promethium, rhenium, rhodium, rubidium, ruthenium, samarium, scandium, selenium, silicon, silver, strontium, tantalum, technetium, tellurium, terbium, thallium, thorium, thulium, tin, titanium, tungsten, vanadium, ytterbium, yttrium, zinc, zirconium, and oxidation states and isotopes thereof; in particular aluminium, antimony, barium, bismuth, calcium, chromium, cobalt, copper, europium, gadolinium, gallium, germanium, gold, indium, iron, lutetium, manganese, magnesium, nickel, osmium, palladium, platinum, rhenium, rhodium, rubidium, ruthenium, samarium, silver, strontium, technetium, terbium, thallium, thorium, tin, yttrium, zinc, and oxidation states or isotopes thereof; in particular calcium, cobalt, copper, europium, gadolinium, gallium, iron,

magnesium, manganese, nickel, palladium, platinum, ruthenium, samarium, thallium, terbium and zinc (and oxidation states or isotopes thereof, preferably cobalt (II, III), copper (I, II), nickel (II, III), zinc (II) and platinum (0, II, V), palladium (0, II, IV), ruthenium (0, II, III, IV, VI, VIII) or isotopes thereof.

As appears from the examples herein, chemical compound having various chelating moieties (Che) containing different spacers (A and B moieties) and functionalities (C moities) can be produced and be tested with different metal ions (e.g. Zn, Cu, Ni, Co, Gd, Mn).

The compounds suitable for use according to the present invention may either be in the form of a chelate or in the form of a chelator. With respect to the latter, a chelate is contemplated to be formed with a metal ion administered together with the chelator of with a metal ion present in the animal to be treated. Thus, a chelator may be administered together with a sufficient amount of a suitable metal ion in the form of e.g. a metal salt, complex or covalent compound.

The chelates or chelators for use according to the invention can be prepared by methods well known to a person skilled in the art. Specific examples are disclosed in the following co-pending Danish patent applications Nos. PA 2001 01032, PA 2001 01033, PA 2001 01034, PA 2000 01035, which are hereby incorporated by reference.

Pharmaceutical compositions

The chelates and chelators for use in the methods according to the invention are normally presented in the form of a pharmaceutical or a cosmetic composition comprising the specific chelate or chelator together with one or more pharmaceutically and/or cosmetically acceptable excipients.

The chelates or chelators may be administered to the animal including a mammal such as, e.g., a human or a domestic animal including horses, pigs, cattle, cats, dogs, sheep by any convenient administration route such as, e.g., the oral, buccal, nasal, ocular, pulmonary, topical, transdermal, vaginal, rectal, parenteral (including *inter alia* subcutaneous, intramuscular, and intravenous), route in a dose that is effective for the individual purposes. A person skilled in the art will know how to chose a suitable administration route.

The effective dosage of a chelate or chelator employed may vary depending on the particular compound employed, the mode of administration, the condition being treated, the age and condition of the animal to be treated and the severity of the condition being treated. Suitable dosages may be ascertained readily by a person skilled in the art.

Suitable dosage forms include powders, granules, granulates, dispersions including solid dispersions, emulsions, including nano-emulsions, suspensions, solutions including solid solutions, mixtures, syrups, drops, aerosols, liniments, ointments, creams, gels including hydrogels, vagitories, suppositories, plasters, patches, tablets, capsules, sachets, troches, devices etc.

The dosage form may be designed to release the chelate or chelator freely or in a controlled manner e.g. with respect to tablets by suitable coatings.

The pharmaceutical or cosmetic compositions may be prepared by any of the method well known to a person skilled in pharmaceutical or cosmetic formulation.

In pharmaceutical or cosmetic compositions, the compounds of Formula I are normally combined with a pharmaceutical excipient, i.e. a therapeutically inert substance or carrier.

The carrier may take a wide variety of forms depending on the desired dosage form and administration route.

The pharmaceutically or cosmetically acceptable excipients may be e.g. fillers, binders, disintegrants, diluents, glidants, solvents, emulsifying agents, suspending agents, stabilizers, enhancers, flavours, colors, pH adjusting agents, retarding agents, wetting agents, surface active agents, preservatives etc. Details can be found in pharmaceutical handbooks such as, .e.g. ., Remington's Pharmaceutical Science or Pharmaceutical Excipient Handbook.

Brief description of the drawings

Figure 1 represents a serpentine model of the MC-1 receptor mutated as described in Example 1. The potential metal-ion binding residues, which can be reached by extracellular acting ligands, are indicated with circles with black letters on gray background.

Figure 2 illustrates the binding and functional properties of the Zn(II). Competition binding studies on the MC-1 and the MC-4 receptor in transiently transfected COS-7 cells using ^{125}I-NDP-α-MSH as a radio-ligand are shown in Figure 2A and 2B, respectively. The dose-response curves for Zn(II) induced [^{3}H]cAMP accumulation measured in transiently transfected COS-7 cells expressing MC-1 and MC-4 receptors are shown in figure 2C and 2D, respectively.

Figure 3 illustrates that mutations of a crucial metal-ion binding residue destroy the agonistic properties of Zn(II). The dose-response curves for Zn(II) induced [^{3}H]cAMP accumulation measured in transiently transfected COS-7 cells expressing wild type MC-1 and the MC-1 receptor with the Cys^{271} mutated into an alanine are shown in Figure 3A. In Figure 3B the α-MSH dose response curve for each of the two construct are shown.

Figure 4 shows a sequence alignment of the five different MC receptors. The amino acid sequence of the five different human MC receptors are aligned using the alignment program Multiple Sequence Alignment *Clustal W1.8.* Equal residues are marked in white on black whereas similar residues are marked in white on gray. The location of the transmembrane segments - TM-I-TM-VII - are indicated in a line above the sequence alignment. The two main residues involved in the metal-ion binding – AspIII:05 (corresponds to Asp119 in the mouse MC-1 receptor which was used for the mutational mapping) and the Cys in extracellular loop III (corresponds to Cys271 in the mouse MC-1 receptor) are indicated by "X"s in this line

Figure 5 illustrates the potentiating and enhancing properties of Zn(II) on the agonist properties of α-MSH peptide. In Figure 5A and 5B [^{3}H]cAMP accumulation is measured in transiently transfected COS-7 cells expressing MC-1 and MC-4 receptors, where a Zn(II) dose-response curve are added on top of a sub-maximal α-MSH stimulation. Figure 5C and 5D shows that the addition of a constant concentration of Zn(II) (10^{-4}M) shifted the dose-response curve of α-MSH and NDP-α-MSH to the left for MC-1R and MC-4R, respectively.

Figure 6 indicates two different binding modes for Zn++ acting either as an agonist alone on an MC receptor (panel A) or acting as a potentiator or enhancer in the presence of an MSH peptide having the important core tetra-peptide sequence – His-Phe-Arg-Trp- (panel B). Note that as an agonist Zn++ binds in between AspIII:05 (Asp119 in mouse MC-1 receptor) and Cys in EC loop III (Cys271 in the mouse MC-1 receptor), which are conserved among all MC receptors, see Fig. 4 (the numbers coresponds to those of the

MC-1 receptor- the numbers differes in the different receptors). As a potentiator or enhancer (panel B), the metal-ion binds in between Cys in EC loop III of the receptor and the His residue of the ligand.

Figure 7 shows examples of the molecular structure of the metal-ion chelating compounds used in Example 3.

Figure 8 shows the correlation between the binding affinities obtained in MC-1 and MC-4 receptor, respectively. The affinities are determined from competition binding studies on the MC-1 and the MC-4 receptor in transiently transfected COS-7 cells using ^{125}I-NDP-α-MSH as a radio-ligand and displaced with various different metal ion chelates. The correlation between the affinities of the chelates obtained in the two different receptors is highly significant ($p < 0.001$).

Figure 9 shows the increased potency and efficacy of the dose response curve of the modified metal ion chelates. The dose-response curves for Zn(II) and Zn(II) chelates induced [^{3}H]cAMP accumulation measured in transiently transfected COS-7 cells expressing MC1 and MC4 receptors are shown in Figure 8A and 8B, respectively.

Figure 10 illustrates that full antagonism was obtained from those metal-ion chelates, which were unable to activate the receptor. [^{3}H]cAMP accumulation measured in transiently transfected COS-7 cells expressing MC-1 receptor, where a Zn(134) dose-response curve is added on top of a sub-maximal α-MSH stimulation.

EXAMPLES

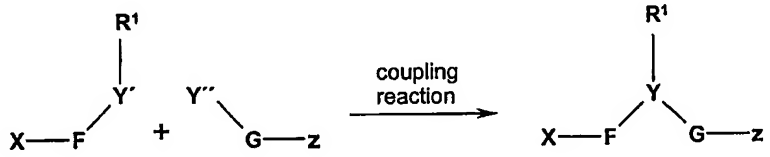
The following examples illustrate the preparation of compounds for use according to the invention

Formula I may be constructed by well-known synthetic steps involving coupling reactions, including Stille-, Suzuki-, Negishi-, Ullmann-couplings (C-C bond formations), condensation reactions, including heterocyclic ring-forming reactions, elimination reactions, cycloaddition reactions, and/or substitution reactions known from the common literature, as illustrated with some typical but non-limiting reaction schemes.

The usual considerations regarding which functional groups that are compatible with the different types of chemistries should always be taken into account when selecting

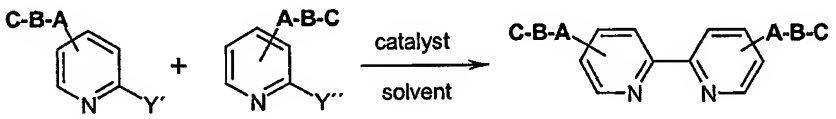
synthetic routes, order of introduction of functional groups and their interconversions, etc, which accordingly will differ on a case by case basis but are evident for the skilled person.

One typical connection of coordinating moieties is depicted in Scheme I, where Y′ and Y′′ are defined such that they represent functional groups enabling coupling reactions.



Scheme I

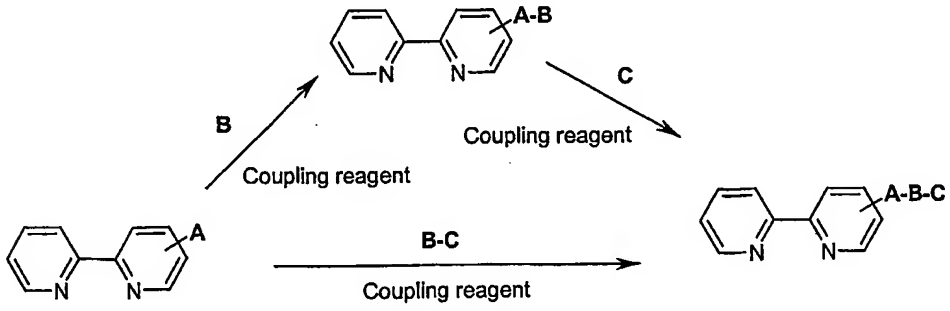
More specific descriptions of the reaction types are exemplified in Schemes II, III and IV respectively. Scheme II illustrates the C-C-bond forming reaction in the 2,2'-bipyridine series.



Reaction type I

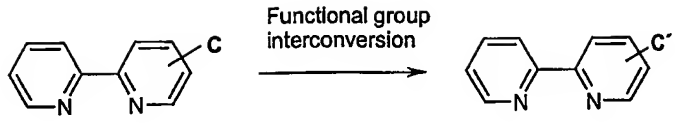
Scheme II

Modification of the chelating scaffolds exemplified by bipyridins can be made in essentially two ways, depicted in Schemes III and IV, either by coupling of an **A**-moiety with a **B**-moiety followed by **C**-moiety, or a **B-C**-moiety, or as illustrated in Scheme IV by a functional group interconversion.



Reaction type II

Scheme III

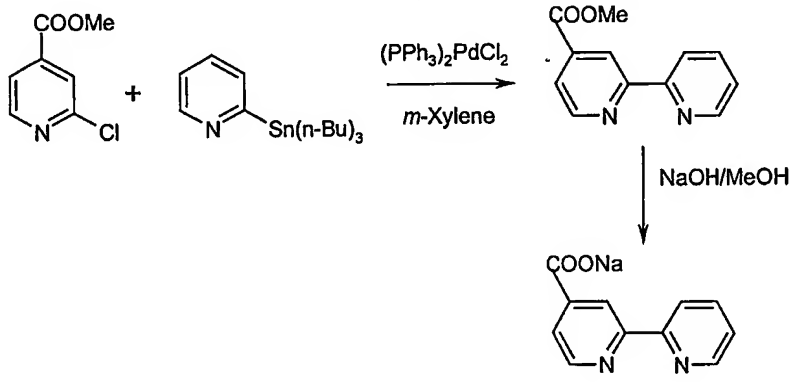


Reaction type III

Where **C** and **C´** represent a change in functionality.

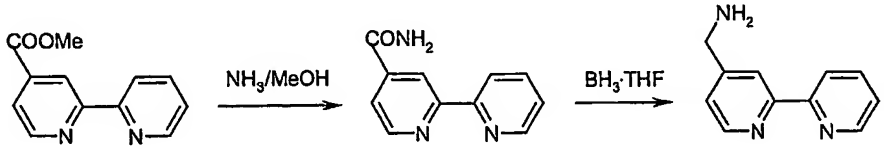
Scheme IV

Coupling of functionalised heterocyclic ring systems such as chloropyridines with trialkyl tin pyridines can be performed by the Stille coupling method, and exemplified in Scheme V.



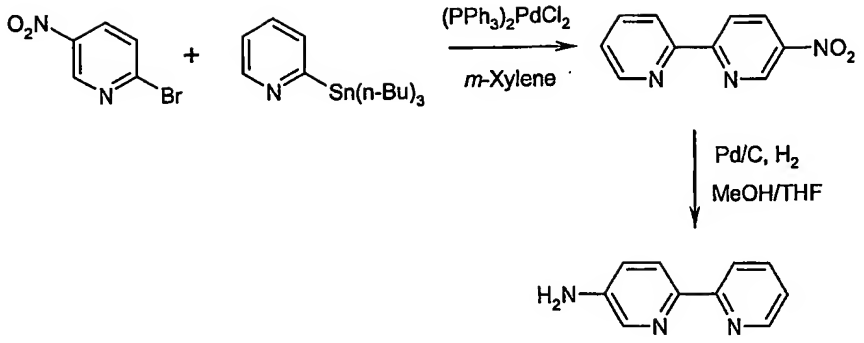
Scheme V

Typical functional group interconversions are exemplified by transforming -$COOCH_3$ into a -CH_2-NH_2 moiety as exemplified with the 2,2'.bipyridine system.



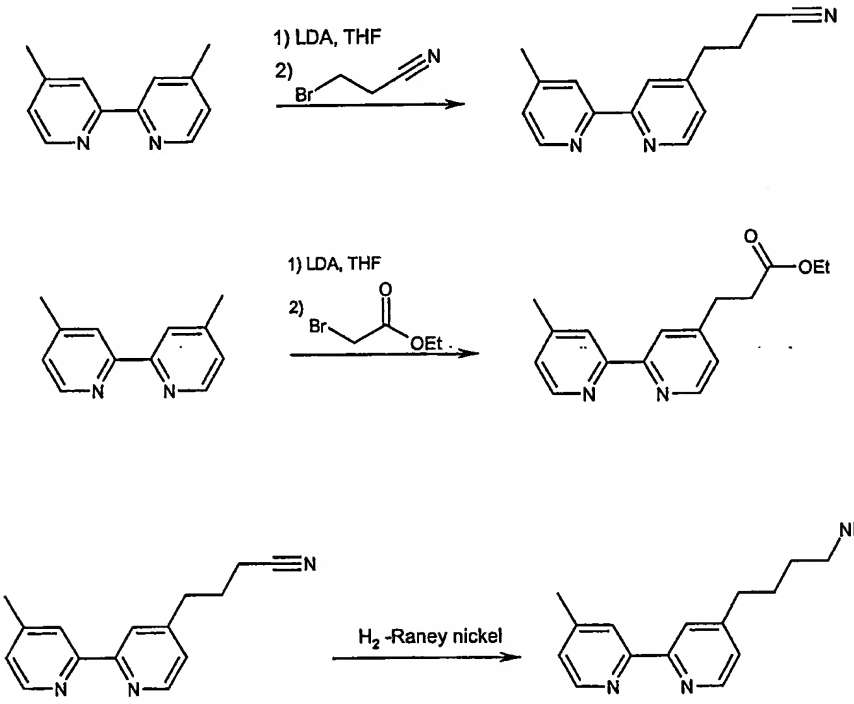
Scheme VI

Certain other types of functionalities on the pyridine ring can accepted in the coupling reaction step as illustrated in Scheme VII.



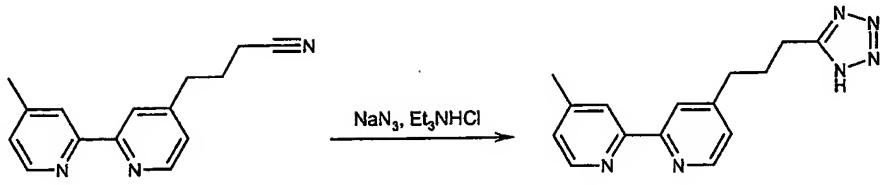
Scheme VII

Other types of functionalisations are illustrated by the synthesis of longer chain 2,2′-bipyridyl amines from the symmetric dimethyl-2,2′- bipyridines, by generation of dimethyl-2,2 bipyridine anion with LDA followed by addition of the appropriate electrophile. Standard reduction of the nitrile yielded the desired product as outlined in Scheme VIII.



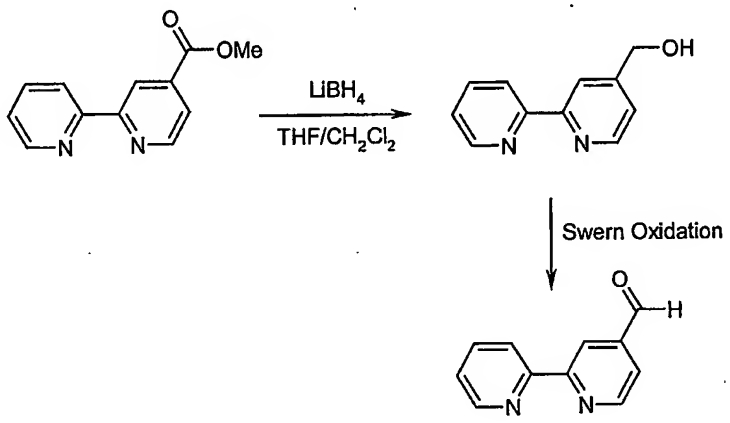
Scheme VIII

Functional group interconversions could utilise common intermediates (cf. Schemes VIII and IX) as illustrated by the bipyridine functionalised chelating scaffold.



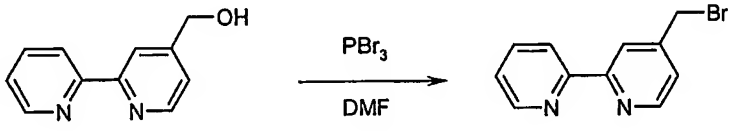
Scheme IX

Reduction of the bipyridine esters were performed by using $LiBH_4$, in DCM/THF as solvent, whereupon the corresponding alcohols were oxidised under Swern conditions to the corresponding aldehydes, as exemplified in Scheme X.



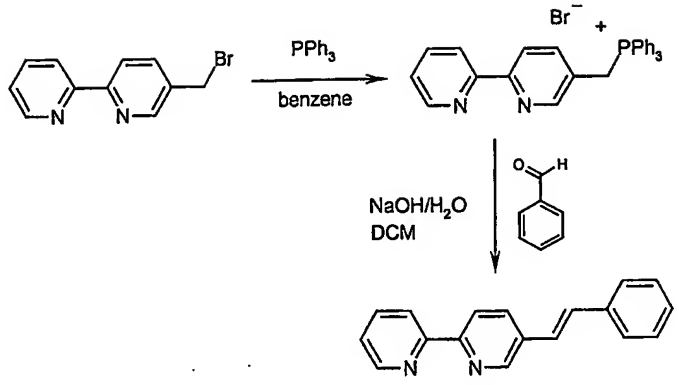
Scheme X

Functional group interconversion of the methylhydroxy functionality to the corresponding bromide can be performed by standard literature procedure as seen in Scheme XI.



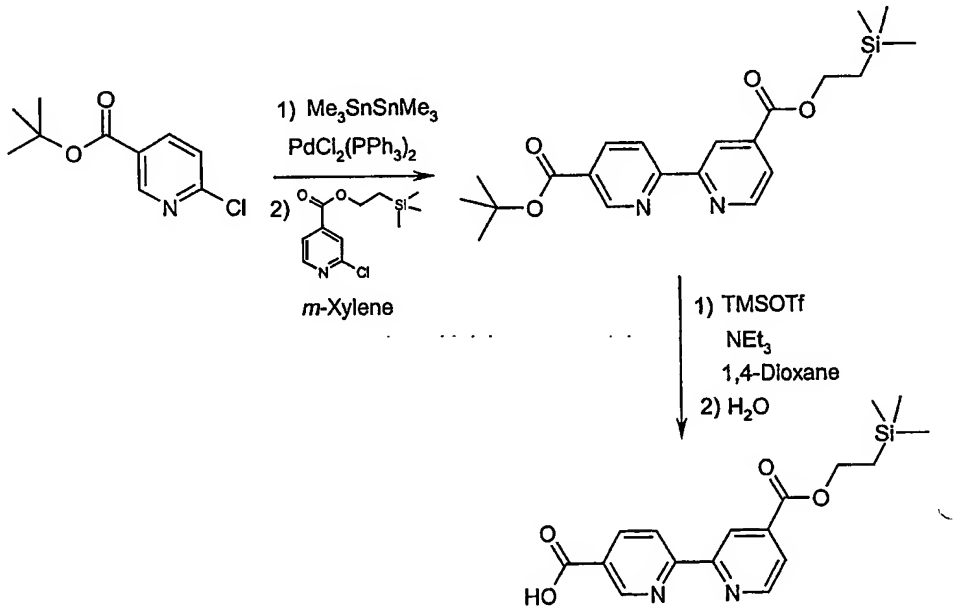
Scheme XI

The synthesis of alkenes the Wittig reaction protocol was utilised as outlined in Scheme XII.



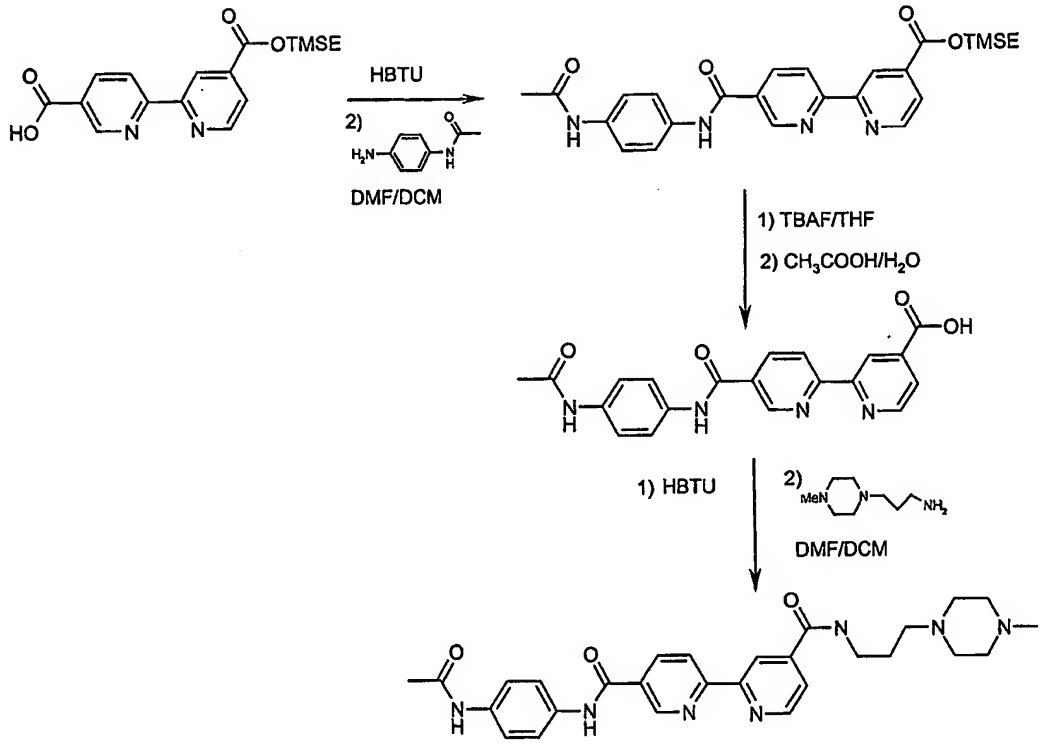
Scheme XII

Coupling of functionalised chloropyridines were performed by using $Me_3SnSnMe_3$, and thereby in situ forming the corresponding trimethyltin pyridine, which was subsequently coupled to the differently substituted chloropyridine as shown in Scheme XIII.



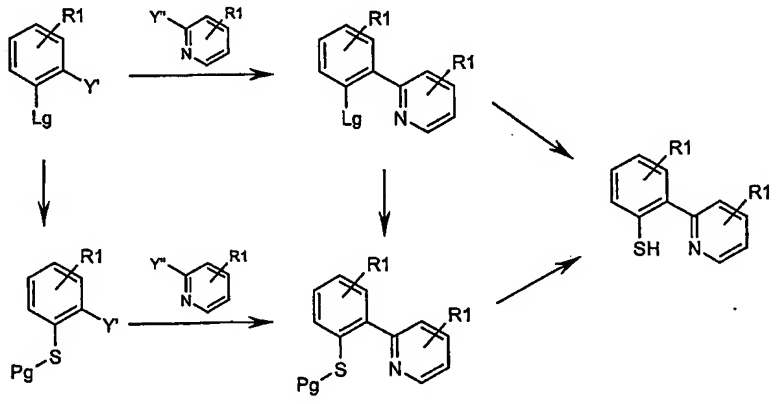
Scheme XIII

Further functionalisations of the unsymmetrically substituted bipyridines were performed by an orthogonal deprotection procedure as in Scheme XIV using standard literature procedure. Amine coupling of the free carboxyl acids can be performed by using a suitable coupling reagent.



Scheme XIV

Similarly, other chelator systems may be formed and manipulated. As an example on a chelator which have one of the coordinating atom(s) outside the ring system is 2-(2-pyridyl)thiophenol (See Scheme XV). In this case, the construction may follow different routes, i.e. the coordinating atoms may be introduced at various stages, protected or unprotected, schematically illustrated in Scheme XV.



Scheme XV

Further functionalisation of the R1-group can be made analogous to the above-described procedures.

Abbreviations.

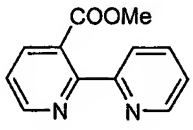
DCM	Dichloromethane
DIBAL	Diisobutylaluminum hydride
DMF	*N,N'*-Dimethylformamide
DMSO	Dimethylsulfoxide
EDC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
HBTU	*O*-Benzotriazole-1-yl-*N,N,N', N'*-tetramethyluronium hexafluorophosphate
HOBT	1-Hydroxybenzotriazole
HPLC	High Performance Liquid Chromatography
LDA	Lithium diisopropylamide
Lg	Leaving group
MS	Mass Spectrometry
NMR	Nuclear magnetic resonance
Pg	Protecting group
R.T.	Room temperature
TBAF	Tetrabutylammonium fluoride
Tf	Triflate
TFFH	Fluoro-*N,N,N'*, -tetramethylformamidinium hexafluorophosphate
THF	Tetrahydrofurane
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl
TMSE	2-(Trimethylsilyl)-ethyl

General chemical procedures

All reagents/chemicals were used as received unless otherwise noted. Methyl esters of 2-chlorocarboxy pyridines were synthesised using carbonyldiimidazole. Coupling of the 2-chloromethyl carboxylates with 2-(tributyl)tin pyridine and hydrolysis of the resulting 2,2'-bipyridinemethyl esters were performed by Stille coupling according to the method

described by Panetta *et al.* (*J. Org. Chem*, **1999**, *64*, 1015-1021). Coupling between the 2,2′-bipyridinesodium carboxylate and selected primary amines (example 3) were perfomed according to standard procedure. Reduction of 4-nitro-2,2′-bipyridine to the corresponding 4-amino-2,2′-bipyridine was accomplished by hydrogenation according to the method by Imperali *et al.* (*J. Org. Chem.*, **1996**, *61*, 8940-8948). All terazoles of 2,2′-bipyridnes were synthesised according to the method of Koguru *et al.* (SYNTHESIS, **1998**, 910-914). Guanidines of amino or alkylamino 2,2′-bipyridine were synthesised according to the method of Patek *et al.* (SYNTHESIS, **1994**, 579-582). Aldehydes of 2,2′-bipyridine were synthesised by reduction of 2,2′-bipyridinemethyl esters using lithiumborohydride according to the method of Uenishi *et al.* (*J. Org. Chem.*, **1993**, *58*, 4382-4388). Oxidation of the resulting methylhydroxy 2,2′-bipyridine to the corresponding aldehyde was performed according to the method of Swern *et al.* (*Tetrahedron*, **1978**, *34*, 1651-1660). All other reactions were carried out according to reported procedures.

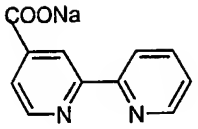
Example 1



2,2′-Methyl-2,2′-bipyridine-3-carboxylate. 2-Chloronicotinic acid methylester (154.9 mmol, 26.7g) was suspended in 500ml dry *m*-xylene, in an oven dried 1000 ml two-necked round bottomed flask equipped with stirrer magnet. 2-Tributyltin pyridine (176.2 mmol, 80g) was added and thereupon *bis*-triphenylphosphinepalladium chloride (9.6 mmol, 6.4g). The resulting mixture was heated to 130 C for 6h under N_2-atmosphere. The dark-brown mixture was then allowed to cool to ambient temperature, and the solvent was removed by evaporation *in vacuo*. The residue was mixed with dichloromethane (50 ml), and purified by column chromatography (CH_2Cl_2:EtOH, 95:5). The pure compound was retrieved as white crystals. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.74 (dd, J = 1.68, 5.1 Hz), 8.61-8.59 (m, 1H), 8.15-8.12 (m, 1H), 7.96 (dd, J = 1.71, 7.8 Hz, 1H), 7.87-7.77 (m, 1H), 7.36 (dd, J = 4.71, 9.0 Hz, 1H), 7.32-7.28 (m, 1H), 3.78 (s, 3H).

In the same manner the corresponding ethyl, propyl, isopropyl, isobutyl, *tert*-butyl, phenyl, pentafluormethyl, 9-fluorenylmethyl, 2-trimethylsilylethyl bipyridine esters (in 3, 4 or 5-position), (alkyl)aldehydes or (alkyl)nitriles are synthesised.

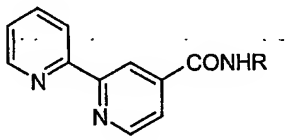
Example 2



Sodium-2,2´-Bipyridine-4-carboxylate. Sodiumhydroxide (131.0 mmol, 5.2g) was dissolved in absolute methanol (300 ml). Bipyridine-4-carboxymethyl ester (130.7 mmol, 28g) was added and the resulting mixture was refluxed for 3h. A white precipitate formed. The mixture was allowed to cool to ambient temperature. The white precipitate was collected by filtration, and washed with ether. The mother liquor diluted with ether (150 ml), and the resulting precipitated was collected by filtration and washed with ether. The remaining solid was allowed to dry at room temperature.

Sodium-2,2´-bipyridine-3-carboxylate and sodium-2,2´-bipyridine-5-carboxylate were prepared according to identical procedure.

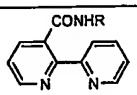
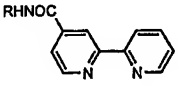
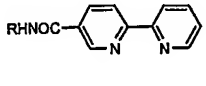
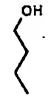
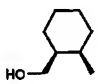
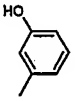
Example 3

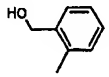
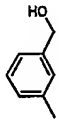
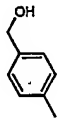
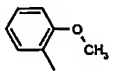
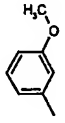
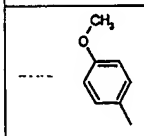
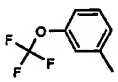
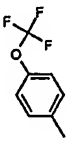
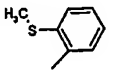


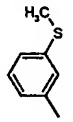
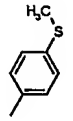
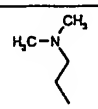
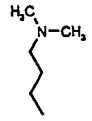
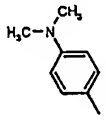
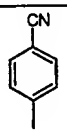
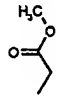
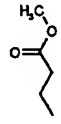
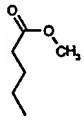
Sodium bipyridinecaboxylate (0.4 mmol, 88.9 mg) was dissolved in 4 ml DMF/CH_2Cl_2 (1:1). Acetic acid (0.4 mmol), coupling reagent (HBTU, TFFH or EDC) (0.4 mmol, 151.7 mg), amine (0.4 mmol) and triethyl amine (0.4 mmol) were added and shaken for 18h at room temperature. The reaction mixture was then quenched with aqueous NaOH (2 ml, 2M), and extracted into 10 ml CH_2Cl_2. The solvent was evaporated at room temperature. Purification was performed either by HPLC (acetonitrile/water in a gradient of acetonitrile in 85 %→ 0 % at a flow rate of 10 ml/min. Column type : YMC-ODS 250x10 mm) or column chromatography on neutral alumina (eluent: acetone/heptane with acetone in a gradient of 20 %→40 %). The desired amides were identified by LC/MS, using 5mM NH_4OAc as the mobile phase, and ES as the ionisation technique.

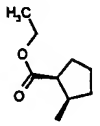
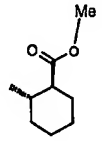
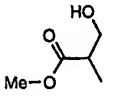
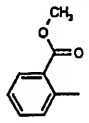
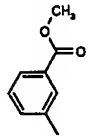
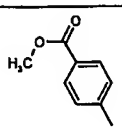
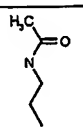
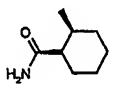
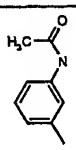
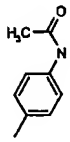
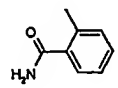
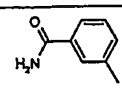
In an analogous manner amides summarised in Table 3-1 are synthesised.

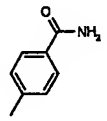
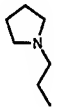
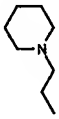
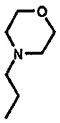
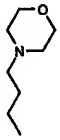
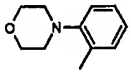
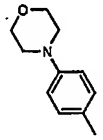
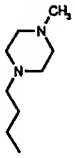
Table 3-1 2,2´-Bipyridyl amides as a typical "A"-type group.

R									
	Coupl. reagent	Analyt. method	Purificat. method	Coupl. reagent	Analyt. method	Purificat. method	Coupl. reagent	Analyt. method	Purificat. method
H	----	NMR	HPLC	HBTU	NMR	HPLC	HBTU	NMR	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
							HBTU	LC-MS	HPLC

				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC			
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC

	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC			
	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	Alumina	HBTU	LC-MS	Alumina	HBTU	LC-MS	HPLC
	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC

				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC						
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC			
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	Alumina	HBTU	LC-MS	HPLC
				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC

	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC	HBTU	LC-MS	Alumina
	TFFH	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
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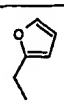
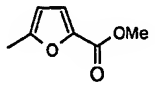
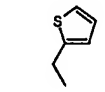
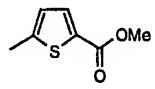
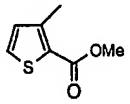
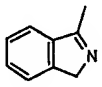
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC			

	TFFH	LC-MS	HPLC						
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	HPLC				HBTU	LC-MS	HPLC
				HBTU	LC-MS	Alumina			
							HBTU	LC-MS	HPLC
				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
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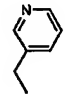
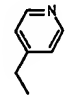
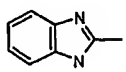
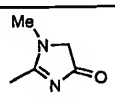
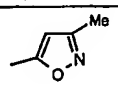
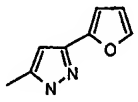
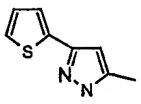
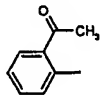
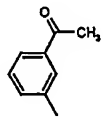
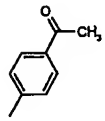
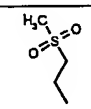
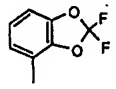
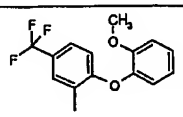
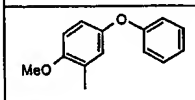
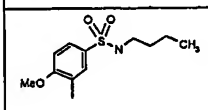
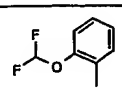
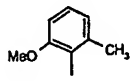
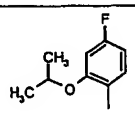
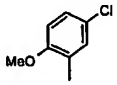
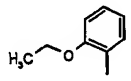
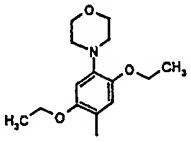
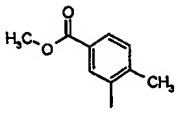
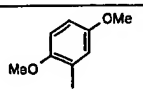
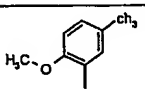
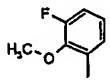
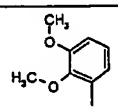
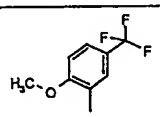
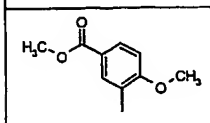
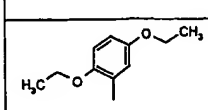
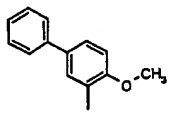
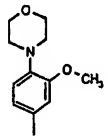
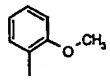
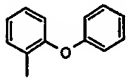
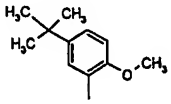
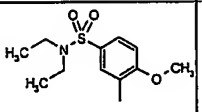
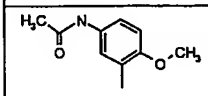
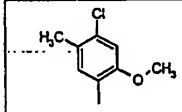
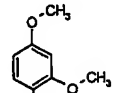
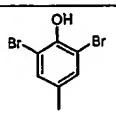
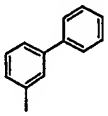
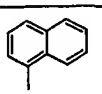
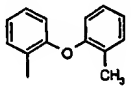
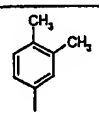
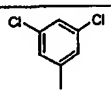
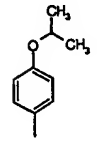
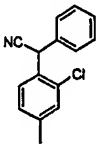
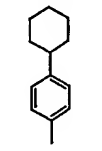
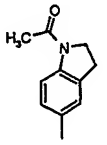
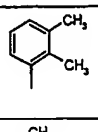
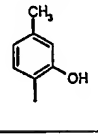
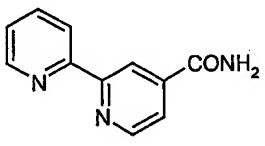
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC			
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
				HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
							HBTU	LC-MS	HPLC

	TFFH	LC-MS	HPLC						
	TFFH	LC-MS	Alumina						
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	Alumina			
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC			
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	TFFH	LC-MS	Alumina	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
	EDC	LC-MS	HPLC	HBTU	LC-MS	HPLC	HBTU	LC-MS	HPLC
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			

				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			

				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			

				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			
				HBTU	LC-MS	HPLC			

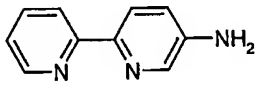


5

2,2´-Bipyridine-4-carboxamide.

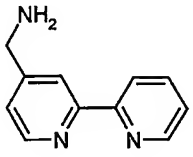
^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.13 (dd, J = 0.75, 2.1 Hz, 1H), 8.72 (ddd, J = 0.96, 5.25, 6.1 Hz, 1H), 8.47-8.42 (m, 2H), 8.36 (dd, J = 3.0, 8.4 Hz, 1H), 8.23 (br. s, 1H), 7.98 (td, J = 1.68, 7.98 Hz, 1H), 7.65 (br. s, 1H), 7.50 (ddd, J = 1.29, 5.1, 7.45 Hz, 1H).

Example 4



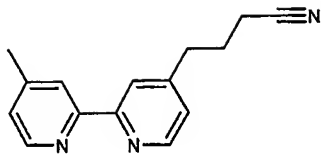
5-Amino-2,2´-bipyridine. 5-Nitro-2,2'-bipyridine (0.641 mol, 129 mg) was dissolved in MeOH/THF (5ml+5ml). To the solution was added Pd/C (5 %, 50 mg) and the reaction mixture was set under an H_2-atmosphere and stirred for 24 h at room temperature. The reaction mixture was filtered through Celite, and the filtrate was evaporated *in vacuo*. The residue was purified by column chromatography (neutral Al_2O_3, 5 % EtOH in DCM), to yield the desired product. Yield: quantitative. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.63-8.61 (m, 1H), 8.27-8.15 (m, 3H), 7.76 (td, J = 1.9, 7.8 Hz, 1H), 7.22 (m, 1H), 7.09 (dd, , J = 2.8, 8.4 Hz, 1H), 3.88 (br. s, 2H).

Example 5



4-(Aminomethyl)-2,2´-bipyridine. 2,2'-Bipyridine-4-carboxamide (15.0 mmol, 3.0g) was placed in an oven dried 100 ml round bottomed flask equipped with stirrer magnet. Borane tetrahydrofurane complex (30 mmol, 30 ml, 1M solution in THF) was slowly added, and the content of the flask was stirred for 15 h. The reaction mixture was quenched with saturated NH_4Cl (aq). The mixture was made basic (pH = 9) with 1M NaOH. The resulting mixture was stirred for 1h at room temperature after which the organic phase was separated, and the aqueous phase was extracted twice with EtOAc (2x30 ml). The combined organic phases were dried over $MgSO_4$, and filtered through a sintered glass funnel. The solvent was removed by evaporation *in vacuo*.

Example 6



4-(3-Cyanopropyl)-4'-metyl-2,2'-bipyridyl: 4,4'-Dimethyl-2,2'-bipyridyl (5.0 g, 27 mmol) was dissolved in dry THF (50 ml) under a nitrogen atmosphere, in a flame-dried flask, equipped with a stirrer. The solution was cooled to –78 °C, and a solution of LDA (20 ml, 33 mmol) was added. The reaction mixture was allowed to warm to room temperature for 1,5 hours. This solution was cannulated into a solution of 3-bromopropionitrile (3.4 ml, 40 mmol) in dry THF (20 ml) at –78 °C, placed in a flame-dried flask under a nitrogen atmosphere, equipped with a stirrer. The reaction mixture was allowed to reach room temperature slowly over night, and quenched by addition of a saturated aqueous solution of sodium bicarbonate. Extractive work-up using ethyl acetate, drying and evaporation, gave the crude product of major components being starting material and expected product. The crude product was purified by column chromatography (Alumina; EtOAc:Heptane 1:2). Yield: 2.1 g (33 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.02 (m, 2H), 2.32 (t, J = 7.07 Hz, 2H), 2.39 (s, 3H), 2.81 (t, J = 7.63 Hz, 2H), 7.10 (m, 2H), 8.20 (s, 1H), 8.24 (s, 1H), 8.47 (d, J = 5.09 Hz, 1H), 8.54 (d, J = 5.09 Hz, 1H).

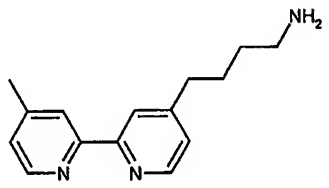
5-(3-Cyanopropyl)-5'-metyl-2,2'-bipyridyl: Same procedure as described above. Yield: 0.67 g (52 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.04 (m, 2H), 2.39 (t, J = 6.97 Hz, 2H), 2.42 (s, 3H), 2.86 (t, J = 7.54 Hz, 2H), 7.68 (d, J = 8.26 Hz, 2H), 8.32 (d, J = 7.91 Hz, 1H), 8.40 (d, J = 8.29 Hz, 1H), 8.53 (s, 2H).

4-(2-Cyanoethyl)-4'-metyl-2,2'-bipyridyl: Same procedure as described above. Yield: 1.17 g (19 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.48 (s, 3H), 2.77 (t, J = 7.35 Hz, 2H), 3.08 (t, J = 7.44 Hz, 2H), 7.22 (m, 2H), 8.30 (s, 1H), 8.37 (s, 1H), 8.56 (d, J = 4.90 Hz, 1H), 8.66 (d, J = 4.98 Hz, 1H).

5-(2-Cyanoethyl)-5'-metyl-2,2'-bipyridyl: Same procedure as described above. Yield: 27 mg (8 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.39 (s, 3H), 2.68 (t, J = 7.25 Hz, 2H), 3.02 (t, J = 7.35 Hz, 2H), 7.59-7.73 (m, 2H), 8.28 (m, 1H), 8.36 (m, 1H), 8.51 (m, 1H), 8.56 (m, 1H).

5-Cyanomethyl-5'-metyl-2,2'-bipyridyl: Same procedure as described above. Yield: 51 mg (15 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.39 (s, 3H), 3.81 (s, 2H), 7.63 (m, 1H), 7.79 (m, 1H), 8.28 (d, J = 8.10 Hz, 1H), 8.40 (d, J = 8.10 Hz, 1H), 8.51 (m, 1H), 8.60 (m, 1H).

Example 7

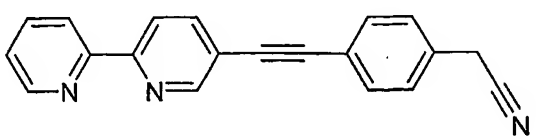


4-(4-Aminobutyl)-4'-methyl-2,2'-bipyridyl: 4-(3-Cyanopropyl)-4'-methyl-2,2'-bipyridyl (125 mg, ca. 0.5 mmol) was dissolved in 96 % ethanol (5 ml) and catalytic amount of Raney nickel was added. The reaction was stirred over night under 1 atmosphere of hydrogen. Evaporated and purified by chromatography (alumina, DCM:MeOH:NH_4OH 95:5:0.5). Yield: 70 mg (58 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.49 (m, 2H), 1.68 (m, 2H), 2.36 (s, 3H), 2.64 (t, J = 7.72 Hz, 2H), 2.68 (s, 2H), 2.70 (t, J = 7.07 Hz, 2H), 7.05 (m, 2H), 8.15 (m, 2H), 8.46 (dd, J = 0.47, 4.99Hz, 1H), 8.48 (dd, J = 0.66, 5.00Hz, 1H).

5-(4-Aminobutyl)-5'-methyl-2,2'-bipyridyl: Same procedure as described above. Yield: 181.4 (44 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.52 (m, 2H), 1.70 (m, 2H), 1.80 (s, 2H), 2.38 (s, 3H), 2.68 (t, J = 7.54 Hz, 2H), 2.74 (t, J = 7.06 Hz, 2H); 7.59 (m, 1H), 7.62 (m, 1H), 8.24 (d, J = 6.03 Hz, 1H), 8.26 (d, J = 8.10 Hz, 1H), 8.48 (s, 1H), 8.49 (s, 1H).

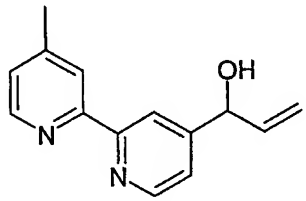
4-(3-Aminopropyl)-4'-methyl-2,2'-bipyridyl: Same procedure as described above. Yield: 190 mg (50 %). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.80 (m, 2H), 2.41 (s, 3H), 2.68 (t, J = 7.16 Hz, 2H), 2.74 (t, J = 7.82 Hz, 2H), 4.48 (s, broad, 2H), 7.28 (m, 2H), 8.23 (s, 1H), 8.25 (s, 1H), 8.53 (d, J = 5.08 Hz, 1H), 8.56 (d, J = 5.08 Hz, 1H).

Example 8



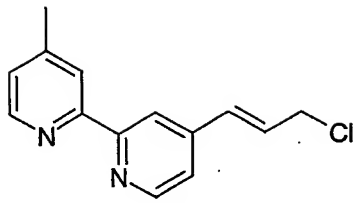
(4-[2,2']Bipyridinyl-5-ylethynyl-phenyl)-acetonitrile. 4-(2-(2'-Pyridyl)pyridyl)acetylene (0.6 g, 2.0 mmol), iodophenylacetonitril (0.54 g, 2.2 mmol), copperiodide (38 mg, 0.2 mmol), tetrakis(triphenylphosphine)palladium(0) (230 mg, 0.2 mmol) and triethylamine (2.8 ml, 20 mmol) in DMF (10 ml) was stirred at R.T. under nitrogen for 24 hours. The reaction was reduced *in vacuo*, and water and ethylacetate added. The organic layer was dried, reduced *in vacuo* and purified on a silica column, using ethylacetate/ether (1:1) as eluent. Recrystalised in ethylacetate. Yield: 30 mg (5 %). ^{1}H NMR($CDCl_3$, 300 MHz) δ 3.82 (s, 1H), 7.35 (m, 1H), 7.38 (d, J = 8.1 Hz, 2H), 7.61 (d, J = 8.1 Hz, 2H), 7.85 (br t, J = 7.7 Hz, 1 H), 7.97 (dd, J = 8.3 Hz, J = 2.1 Hz, 1H), 8.45 (d, J = 8.1 Hz, 2H), 8.72 (d, J = 4.9 Hz, 1H), 8.85 (m, 1H).

Example 9



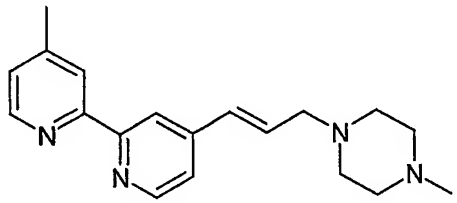
1-(4'-Methyl-[2,2']bipyridinyl-4-yl)-prop-2-en-1-ol. 4-Formyl-4'-methyl-2,2'-bipyridine (10.1 mmol, 2.0 g) was dissolved in dry tetrahydrofuran (100 ml) at -20 °C before vinyl magnesium bromide (12.0 mmol, 1M, 12.0 ml) was added dropwise. The reaction mixture was stirred for 2h before a saturated aqueous solution of ammonium chloride (50 ml) was added. The resulting mixture was extracted with ethyl acetate (3 x 100 ml); the organics were combined, washed with brine (100 ml), dried and evaporated. Purification by column chromatography (40 % [10 % Et_3N in EtOAC]/petrol) yielded the *alcohol* as an orange solid. Yield 63 %, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.56 (d, 1H, J = 5.1 Hz), 8.48 (d, 1H, J = 5.1 Hz), 8.34 (m, 1H), 8.17 (m, 1H), 7.29 (m, 1H), 7.08 (m, 1H), 5.98 (ddd, J = 16.0, 10.0, 3.9 Hz, 1H), 5.34 (dt, J = 16.0, 1.3 Hz, 1H), 5.23 (br. d, J = 16.0 Hz, 1H), 5.17 (dt, J = 10.0, 1.3 Hz, 1H), 2.39 (s, 3H).

Example 10



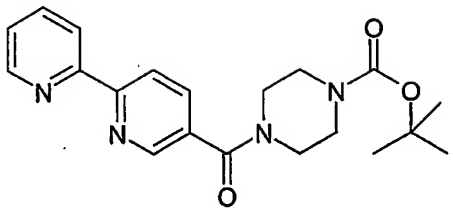
4'-(3-Chloro-propenyl)-4-methyl-[2,2']bipyridinyl. The alcohol from Example 9 (6.3 mmol, 1.5 g) was dissolved in dry dichloromethane (30 ml) and stirred at 0 °C before thionyl chloride (30 ml) was added in one portion. The reaction was stirred until tlc showed consumption of all starting material. The reaction mixture was allowed to warm to room temperature before careful addition of water (50 ml) and sodium bicarbonate (50 ml). The mixture was then extracted with dichloromethane (3 x 100 ml); the combined organics were dried over silica and then filtered through a plug of celite before being concentrated *in vacuo* to yield a yellow oil which was used without purification.

Example 11



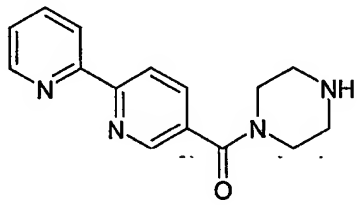
4-Methyl-4'-[3-(4-methyl-piperazin-1-yl)-propenyl]-2,2'-bipyridine. The chloride from Example10 (1.32 mmol, 0.342 g) was dissolved in dry dichloromethane (25 ml) at ambient temperature. Piperazine (13.2 mmol, 1.13 g) was added and the solution was stirred overnight. The reaction mixture was extracted with hydrochloric acid (3 x 20 ml, 1M). The combined aqueous were washed with dichloromethane (10 ml), basified to pH 10 and extracted with dichloromethane (3 x 50 ml). The combined organics were washed with brine (50 ml) dried over sodium sulphate and then concentrated *in vacuo*. chromatography (10 % MeOH/DCM) yielded the *amine* as a mixture of geometric isomers. Yield 66 %, ^{1}H NMR ($CDCl_3$, 300 MHz) (major isomer reported): δ 8.53 (d, J = 5.1 Hz, 1H), 8.46 (d, J = 5.1 Hz, 1H), 8.31 (s, 1H), 8.16 (s, 1H), 7.18 (m, 1H), 7.06 (m, 1H), 6.65-6.48 (m, 2H), 3.14 (m, 2H), 2.60-2.40 (br. s, 8H), 2.38 (br. s, 3H), 2.24 (br. s, 3H).

Example 12



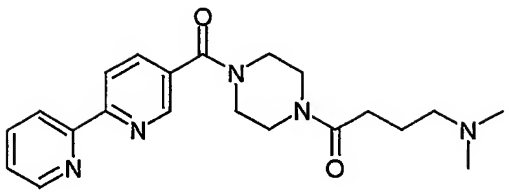
4-([2,2']Bipyridinyl-5-carbonyl)-piperazine-1-carboxylic acid tert-butyl ester. To a dry mixture of *N*-butoxycarbonyl piperazine (5 mmol, 1.1 g), 2,2'-bipyridyl-4-carboxylic acid (5 mmol, 1.0 g), EDC (6.5 mmol, 1.25 g) and hydroxybenzotriazole monohydrate (6.0 mmol, 0.81 g) was added dry dichloromethane (50 ml). The mixture was stirred at ambient temperature for 16 h before being washed with a saturated solution of sodium bicarbonate (10 ml), water (10 ml), brine (10 ml), dried over sodium sulphate and condensed *in vacuo*. The product was used without further manipulation. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 8.72 (m, 2H), 8.50 (d, J = 8.0 Hz, 1H), 8.43 (d, J = 8.0 Hz, 1H), 7.88 (m, 2H), 7.36 (m, 1H), 3.90-3.25 (m, 8H), 1.52 (s, 9H).

Example 13



[2,2']Bipyridinyl-5-yl-piperazin-1-yl-methanone. The product of example 12 (0.19mmol, 73 mg) was dissolved in dichloromethane (5 ml) at ambient temperature. Trifluoroacetic acid (1 ml) was added and stirring continued for 1 h. The reaction mixture was washed with water (2 x 10 ml) and the combined aqueous basified to pH 10 before being extracted with dichloromethane (2 x 10 ml). The combined organics were washed with brine, dried over sodium sulphate and concentrated in *vacuo* to give the amine. Yield: 47 %. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.72 (m, 2H), 8.48 (d, J = 8.1 Hz, 1H), 8.42 (d, J = 8.1 Hz, 1H), 7.85 (m, 2H), 7.35 (dd, J = 7.9, 4.9 Hz, 1H), 3.90-3.40 (br m, 4H), 3.50- 2.80 (br m, 4H).

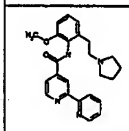
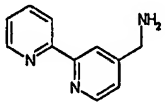
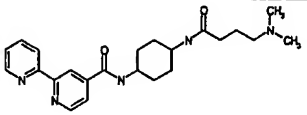
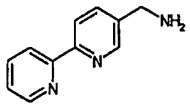
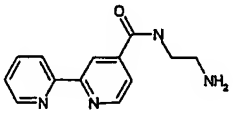
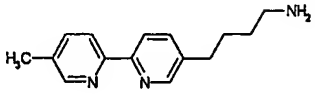
Example 14

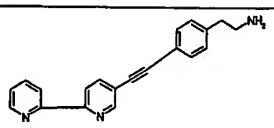
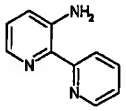
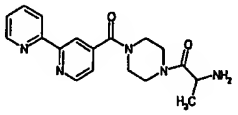
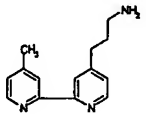
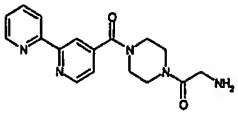
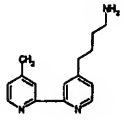
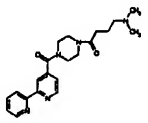
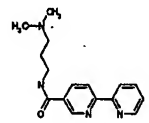
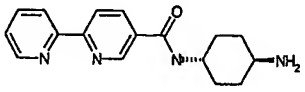
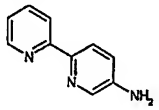
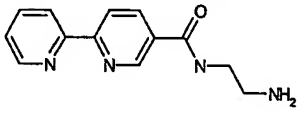
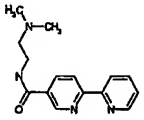
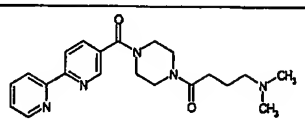
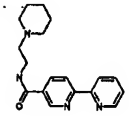
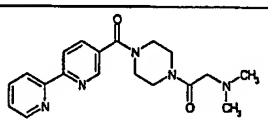
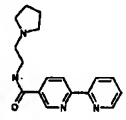
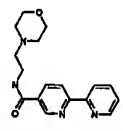
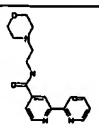
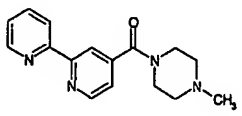
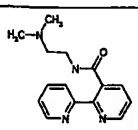


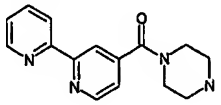
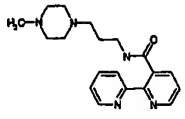
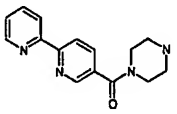
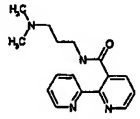
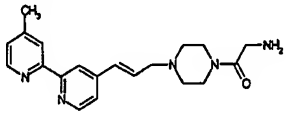
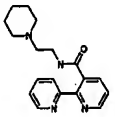
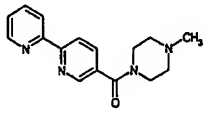
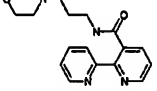
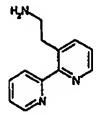
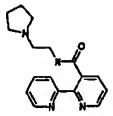
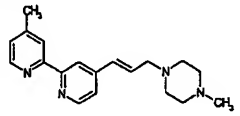
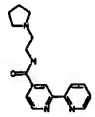
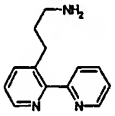
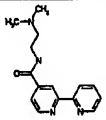
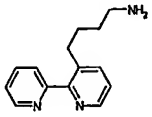
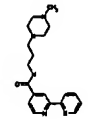
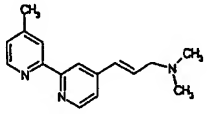
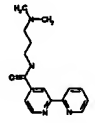
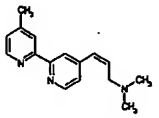
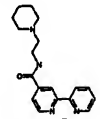
1-[4-([2,2']Bipyridinyl-5-carbonyl)-piperazin-1-yl]-4-dimethylamino-butan-1-one. A screw-top vial was charged with PS-carbodiimide resin (200mg) followed by a solution of γ-dimethylaminobutyric acid (0.15mmol, 25 mg) in dichloromethane (1 ml). The suspension was stirred gently for 5 min. before the addition of a solution of the amine from Example 13 (0.1mmol, 25 mg) in dichloromethane (1 ml). Stirring continued for 16 h before the addition of PS-trisamine (200 mg) and further stirring for 2 h. The solids were removed by filtration and the residue washed with dichloromethane (10 ml). The combined organics were dried in vacuo to give the tertiary amine. Yield: 41 mg (99 %). 1H NMR (CDCl3,300 MHz): δ 8.70 (m, 2H), 8.45 (d, J = 8.1 Hz, 1H), 8.40 (d, J = 8.1 Hz, 1H), 7.85 (m, 2H), 7.38 (dd, J = 4.9, 4.7 Hz, 1H), 3.95- 3.45 (m, 8H), 3.35 (t, J = 7.2 Hz, 2H), 2.80 (s, 6H), 2.68 (t, J = 6.5 Hz, 2H), 2.54 (app. q, J = 7.0 Hz, 2H).

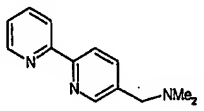
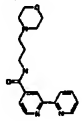
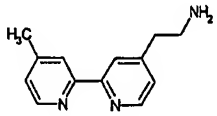
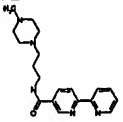
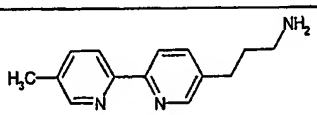
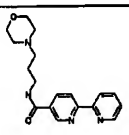
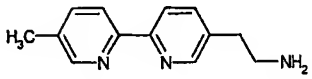
In analogous manners 2,2´-bipyridyl amines are synthesised according to table 14-1, giving an representative example for a "C"-type group.

Table 14-1 2,2´-Bipyridyl amines in a "C"-type group.

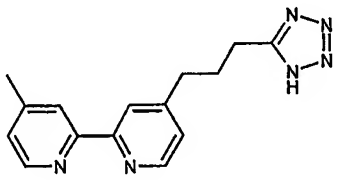
Structure	Method	Structure	Method
	Ex 3		Ex 5
	Ex 14		Ex 5
	Ex 13		Ex 7

	Ex 7		Ex 4
	Ex 14		Ex 7
	Ex 14		Ex 7
	Ex 14		Ex 3
	Ex 13		Ex 4
	Ex 13		Ex 3
	Ex 14		Ex 3
	Ex 14		Ex 3
	Ex 3		Ex 3
	Ex 13		Ex 3

	Ex 13		Ex 3
	Ex 13		Ex 3
	Ex 14		Ex 3
	Ex12		Ex 3
	Ex 7		Ex 3
	Ex 11		Ex 3
	Ex 7		Ex 3
	Ex 7		Ex 3
	Ex 11		Ex 3
	Ex 11		Ex 3

	Ex 11		Ex 3
	Ex 7		Ex 3
	Ex 7		Ex 3
	Ex 7		

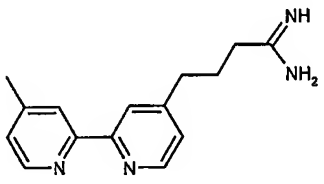
Example 15



4'-Methyl-4-[3-(1H-tetrazol-5-yl)-propyl]-2,2'-bipyridine. 4-(3-Cyanopropyl)-4'-methyl-2,2'-bipyridyl (0.72 g, 3 mmol) was dissolved in dry toluene (10 ml), followed by addition of sodium azide (0.6 g, 9 mmol) and triethylammonium chloride (1.25 g, 9 mmol). The reaction was heated to 100 °C for 18 hours. After cooling, a small amount of water is added, the phases separated, and the aqueous phase acidified with hydrochloric acid. The crude product precipitated as a red oil, which is purified on a column (silica, EtOAc:MeOH 1:2). Yield: 0.6 g (71 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.13 (m, 2H), 2.41(s,

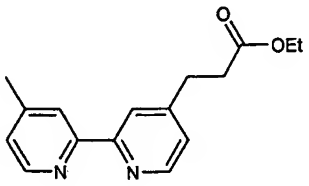
3H), 2.69 (t, J = 7.35 Hz, 2H), 2.92 (t, J = 7.45 Hz, 2H), 5.79 (s, broad, 1H)), 7.08 (m, 1H), 7.14 (m, 1H), 8.02 (s, 1H), 8.07 (s, 1H), 8.46 (d, J = 5.27 Hz, 1H), 8.48 (d, J = 5.09 Hz, 1H).

Example 16



4-(4-Butyramidine)-4'-methyl-2,2'-bipyridine. Dry NH_4Cl (0.17g, 3mmol) in dry toluene (3 ml) was stirred at an ice-bath under nitrogen, and trimethylaluminium (1.6 ml, 2.0M, 3.2 mmol) added slowly. The mixture was allowed to attain room temperature. 4-(3-Cyanopropyl)-4'-methyl-2,2'-bipyridine (0.25 g, 1 mmol) was added, and the reaction is heated to 90 °C for 3 days. Alumina (9 g) was suspended in chloroform (40 ml), and the reaction mixture poured into it, followed by methanol (50 ml), and the reaction mixture was stirred for 0.5 hours. The slurry was filtered and concentrated *in vacuo*. Extractive work-up in DCM and aqueous $NaHCO_3$. Purification on alumina column (heptane:ethylacetate:ethanol (2:2:1)). Yield: 0.05 g (18 %). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.85 (p, J = 7.7 Hz, 2H), 2.10 (t, J = 7.7 Hz, 2H), 2.42 (s, 3H), 2.68 (t, J = 7.7 Hz, 2H), 6.74 (br. s, 1H), 7.28 (m, 2H), 8.23 (m, 2H), 8.55 (m, 2H).

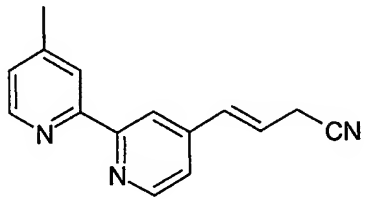
Example 17



Ethyl 4-(3-carboxypropyl)-4'-metyl-2,2'-bipyridyl: 4,4'-Dimethyl-2,2'-bipyridyl (2.5 g, 13.5 mmol) was dissolved in dry THF (20 ml) under a nitrogen atmosphere, in a flame-dried flask, equipped with a stirrer. The solution was cooled to –78 °C, and a solution of LDA

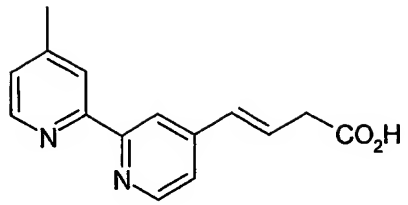
(10 ml, 16.8 mmol) was added. The reaction mixture was allowed to warm to room temperature for 1,5 hours. This solution was cannulated into a solution of ethyl 2-bromoacetate (2.3 ml, 20 mmol) in dry THF (15 ml) at –78 °C, placed in a flame-dried flask under a nitrogen atmosphere, equipped with a stirrer. The reaction mixture was allowed to reach room temperature slowly over night, and quenched by addition of a saturated aqueous solution of sodium bicarbonate. Extractive work-up using ethyl acetate, drying and evaporation, gave the crude product. Purified by column chromatography (Silica; DCM:MeOH:NH_4OH 95:5:0.5). Yield: 1.86 g (51 %). ^1H NMR ($CDCl_3$, 300 MHz) δ 1.17 (t, J = 7.16 Hz, 3H), 2.40 (s, 3H), 2.66 (t, J = 7.63 Hz, 2H), 2.99 (t, J = 7.63 Hz, 2H), 4.07 (q, J = 7.16 Hz, 2H), 7.13 (m, 2H), 8.22 (s, 1H), 8.28 (s, 1H), 8.49 (d, J = 5.08 Hz), 8.52 (d, J = 5.09 Hz, 1H).

Example 18



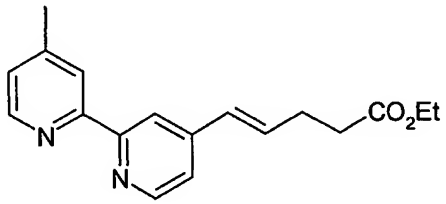
4-(4'-Methyl-[2,2']bipyridinyl-4-yl)-but-3-enenitrile. The chloride from Example 10 (3.5 mmol, 0.868g) was dissolved in dry ethanol (40 ml) at ambient temperature. Potassium cyanide (4.3 mmol, 0.209g) dissolved in water (3.5 ml) was added in one portion and the resulting solution was heated to 78 °C for 15 h. The solvent was then removed *in vacuo* before the crude product was subjected to column chromatography (40 % [10 % Et_3N in EtOAC]/petrol) yielded the cyanide as an orange solid. Yield: 18 %, ^1H NMR ($CDCl_3$, 300 MHz): δ 8.65 (d, J = 5.1 Hz, 1H), 8.55 (d, J = 5.1 Hz, 1H), 8.43 (s, 1H), 8.24 (s, 1H), 7.29 (dd, J = 4.9, 0.8 Hz, 1H), 7.16 (dd, J = 4.9, 0.8 Hz, 1H), 6.80-6.55 (m, 2H), 3.78 (d, J = 7.2 Hz, 2H), 2.45 (s, 3H).

Example 19



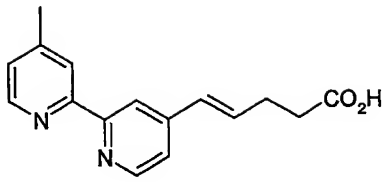
4-(4'-Methyl-[2,2']bipyridinyl-4-yl)-but-3-enoic acid. The cyanide from Example 18 (0.48 mmol, 0.112g) was taken up in sodium hydroxide solution (20 mmol, 4M, 5 ml) and stirred for 15h at reflux. The solvent was then remove *in vacuo* and the product purified on chromatotron (EtOAc,/petrol gradient). Yield 3.2 %, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.62 (d, J = 5.1 Hz, 1H), 8.55 (d, J = 5.1 Hz, 1H), 8.39 (s, 1H), 8.25 (s, 1H), 7.27 (m, 1H), 7.16 (m, 2H), 6.71 (m, 1H), 4.41 (m, 2H), 2.46 (s, 3H).

Example 20



5-(4'-Methyl-[2,2']bipyridinyl-4-yl)-pent-4-enoic acid ethyl ester. The alcohol from Example 9 (0.64 mmol, 0.144g) was dissolved in triethyl orthoacetate (2 ml), and toluene (8 ml) under a nitrogen atmosphere. Acetic acid (20 μl) was added and the resulting solution was heated to 120 °C for 3 h. After cooling to ambient temperature, a saturated solution of sodium carbonate (10 ml) was added and the mixture extracted with ethyl acetate (3 x 20 ml). The combined organics were washed with brine (50 ml) before the addition of petrol (120 ml). The organic solution was passed through a plug of silica and the filtrate reduced *in vacuo*. Purification by column chromatography (20 % [10 % Et_3N in EtOAC]/petrol) yielded the *ester* as an pale yellow solid. Yield: 40 %.

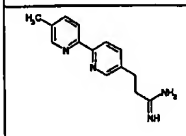
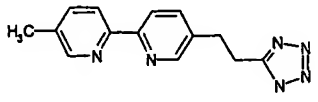
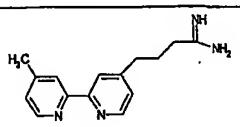
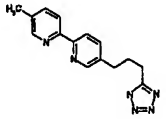
Example 21

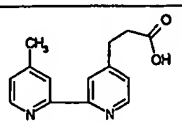
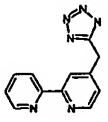
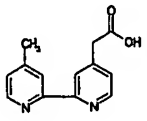
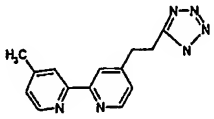
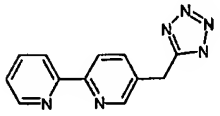
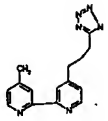
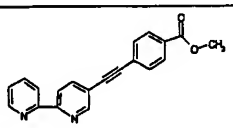
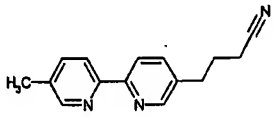
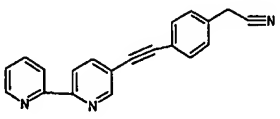
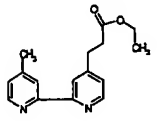
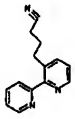
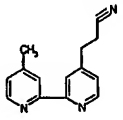
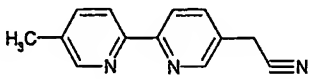
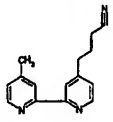
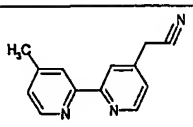
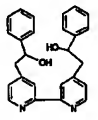
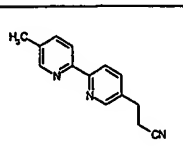
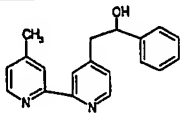


5-(4'-Methyl-[2,2']bipyridinyl-4-yl)-pent-4-enoic acid. Ethyl ester from Example 20 (0.045 mmol, 0.020g) was taken up in a mixture of THF (0.5 ml), ethanol (0.5 ml) and water (0.1 ml) at ambient temperature before potassium carbonate (0.045 mmol, 0.063 g) was added and the resulting suspension stirred overnight. Barium hydroxide (0.1 g) was added and the suspension stirred for a further 7 h before the pH of the mixture was adjusted to 5 and the mixture extracted with ethyl acetate (3 x 5 ml). The combined organics were dried and reduced *in vacuo*. Yield: 50 %, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.60 (m, 2H), 8.33 (s, 1H), 8.24 (s, 1H), 7.21 (dd, J = 6.1, 4.9 Hz, 1H), 6.66 (m, 1H), 6.51 (d, J = 15.8 Hz, 1H), 2.62 (m, 4H), 2.46 (s, 3H).

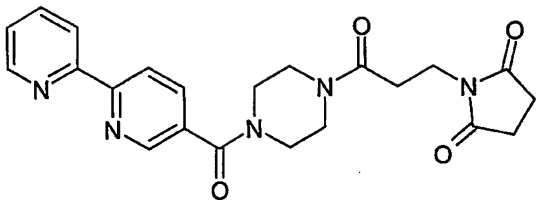
In analogous manners the following functional groups were introduced to the 2,2´-bipyridine scaffold, shown in table 21-1, giving an representative example for various kinds of "C"-type groups.

Table21-1 Various functionalized 2,2´-bipyridines as part of various "C"-type groups.

Structure	Method	Structure	Method
	Ex 16		Ex 15
	Ex 16		Ex 15

	Ex 21		Ex 15
	Ex 21		Ex 15
	Ex 15		Ex 15
	Ex 8		Ex 6
	Ex 8		Ex 17
	Ex 6		Ex 6
	Ex 6		Ex 6
	Ex 6		Ex 17
	Ex 6		Ex 17

Example 22



1-{3-[4-([2,2']Bipyridinyl-5-carbonyl)-piperazin-1-yl]-3-oxo-propyl}-pyrrolidine-2,5-dione. The corresponding maleimide (made analogously to Example 14) (0.11mmol, 43 mg) was dissolved in methanol (5 ml) at ambient temperature before 10 % palladium on carbon (10 mg) was added and the atmosphere exchanged first with nitrogen and second with hydrogen. The suspension was stirred vigorously for 16 h and then the reaction mixture was filtered through a plug of celite. The residue was washed with methanol (50 ml). The combined organics were evaporated to dryness to give the succinimide. Yield: 99 %. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.72 (m, 2H), 8.46 (d, J = 8.1 Hz, 1H), 8.39 (d, J = 8.1 Hz, 1H), 7.87 (m, 2H), 7.36 (m, 1H), 3.90-3.40 (m, 10H), 2.70 (m, 6H).

By the same procedure the following compound was made from the unsaturated amide:

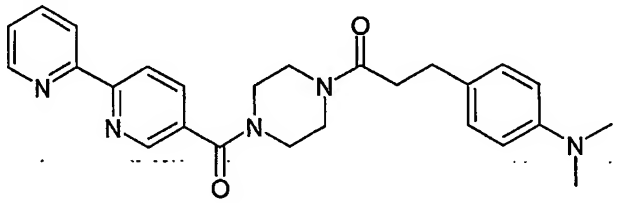
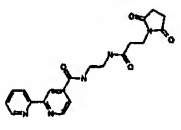
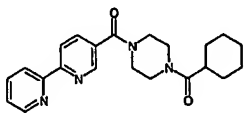
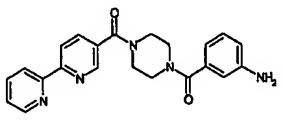
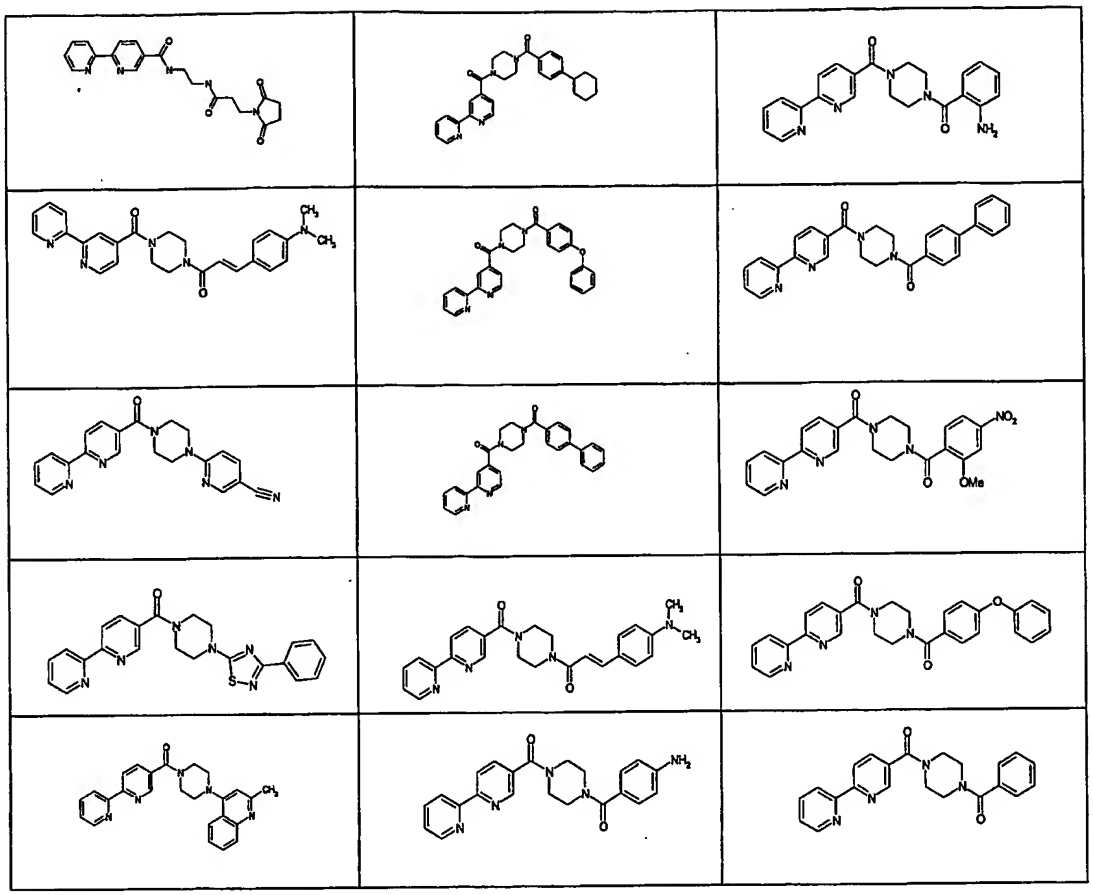
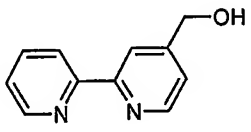


Table 22-1 2,2'-Bipyridines with various C-groups in a typical "A"-type group made similarly to Example 14.



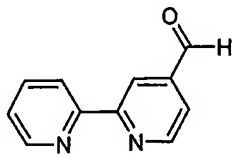
Example 23



4-(Hydroxymethyl)-2,2´-bipyridine. Methyl 2,2´-bipyridine-4-carboxylate (9.34 mmol, 2.0 g) was dissolved in MeOH/DCM (5 ml/50 ml), whereupon $LiBH_4$ (18.67 mmol, 0.4 g, 2 equiv.) was added and the reaction mixture was stirred at room temperature for 3h. Another portion of $LiBH_4$ (9.33 mmol, 0.2 g, 1 equiv.) was added and the reaction mixture was stirred at room temperature for 16 h. The reaction was quenched with acetone. The solvent was removed *in vacuo* after which the solid residue was dissolved in DCM, and

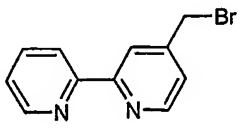
chromatographed on a silica column (DCM/MeOH/NH_3, 100/10/1).Yield: 92 %. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.59 (ddd, *J*= 0.93, 1.5, 3.96 Hz, 1H), 8.52 (dd, *J*= 0.57, 2.25 Hz, 1H), 8.29 (dt, *J*= 0.96, 8.07 Hz, 1H), 8.26-8.25 (m, 1H), 7.77 (td, *J*= 1.86, 7.80 Hz, 1H), 7.29-7.23 (m, 2H), 4.71 (s, 2H), 4.28 (br. s, 1H).

Example 24



4-(Carboxaldehyde)-2,2′-bipyridine. Oxalyl chloride (23.64 mmol, 2.1ml, 1.5 equiv.) was dissolved in dry DCM (30 ml) and cooled to –78 °C. DMSO (31.5 mmol, 2.2 ml, 2 equiv.) was dissolved in DCM (15 ml) and was thereafter added dropwise to the oxalyl chloride solution. The 4-(methylhydroxy)-2,2′-bipyridine (15.76 mmol, 2.0g) dissolved in DCM (15 ml) was then added, and the mixture was stirred at -78 °C for 5 h under an N_2-atmosphere. Triethyl amine (78.8 mmol, 11.0 ml, 5 equiv.) was then added and the reaction mixture was allowed to warm to ambient temperature. DCM (100 ml) was added and sat. $NaHCO_3$ (150 ml) was added. The organic phase was separated and the aqueous phase was extracted with DCM (2x100 ml). The combined organic phases were dried over $MgSO_4$, and the solvent was evaporated *in vacuo* The crude product was purified by column chromatography (DCM/MeOH/NH_3, 100/10/1). Yield: 44 %. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.18 (s, 1H), 8.90-8.83 (m, 1H), 8.73-8.70 (m, 2H), 8.50-8.39 (m, 1H), 7.86 (td, *J*= 1.71, 7.5 Hz, 1H), 7.73 (dd, *J*= 1.53, 4.95 Hz, 1H), 7.39-7.34 (m, 1H).

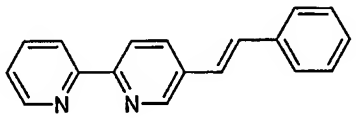
Example 25



4-(Bromomethyl)-2,2′-bipyridine. 4-(Hydroxymethyl)-2,2′-bipyridine (5.37 mmol, 1.0 g) was dissolved in DMF (15 ml). PBr_3 (5.37 mmol, 0.5 ml) was added dropwise at room temperature under inert atmosphere. The reaction mixture was stirred at room

temperature for 15 h. Water (50 ml) was added as the reaction vessel was cooled on an ice-bath. Ethyl acetate (100 ml) was added, and sat. $NaHCO_3$ (100 ml) was added. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (2x50 ml). The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated *in vacuo*. Column chromatography of the crude material (DCM/MeOH, 100/10) yielded the pure bromo methyl compound. Yield. 60 %. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.69-8.66 (m, 2H), 8.44-8.41 (m, 2H), 7.84 (td, J= 1.86, 7.5 Hz, 1H), 7.36-7.31 (m, 2H), 4.49 (s, 2H).

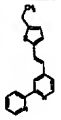
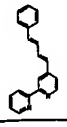
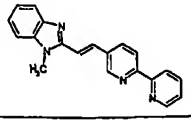
Example 26

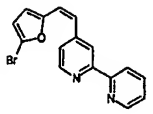
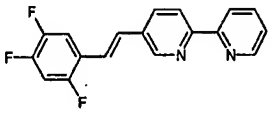
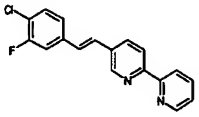
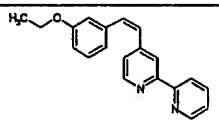
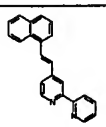
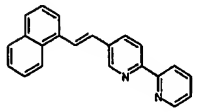
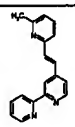
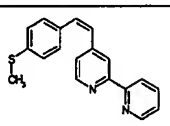
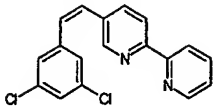
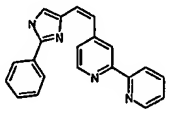
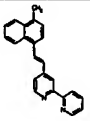
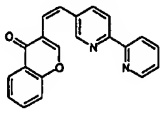
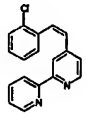
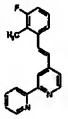
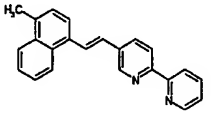
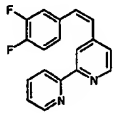
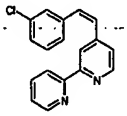
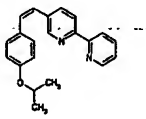
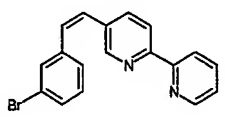
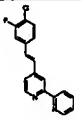
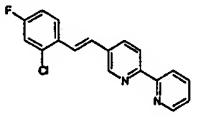
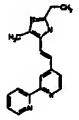
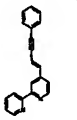
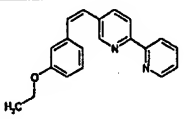
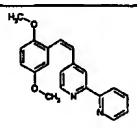
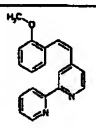
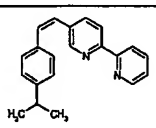


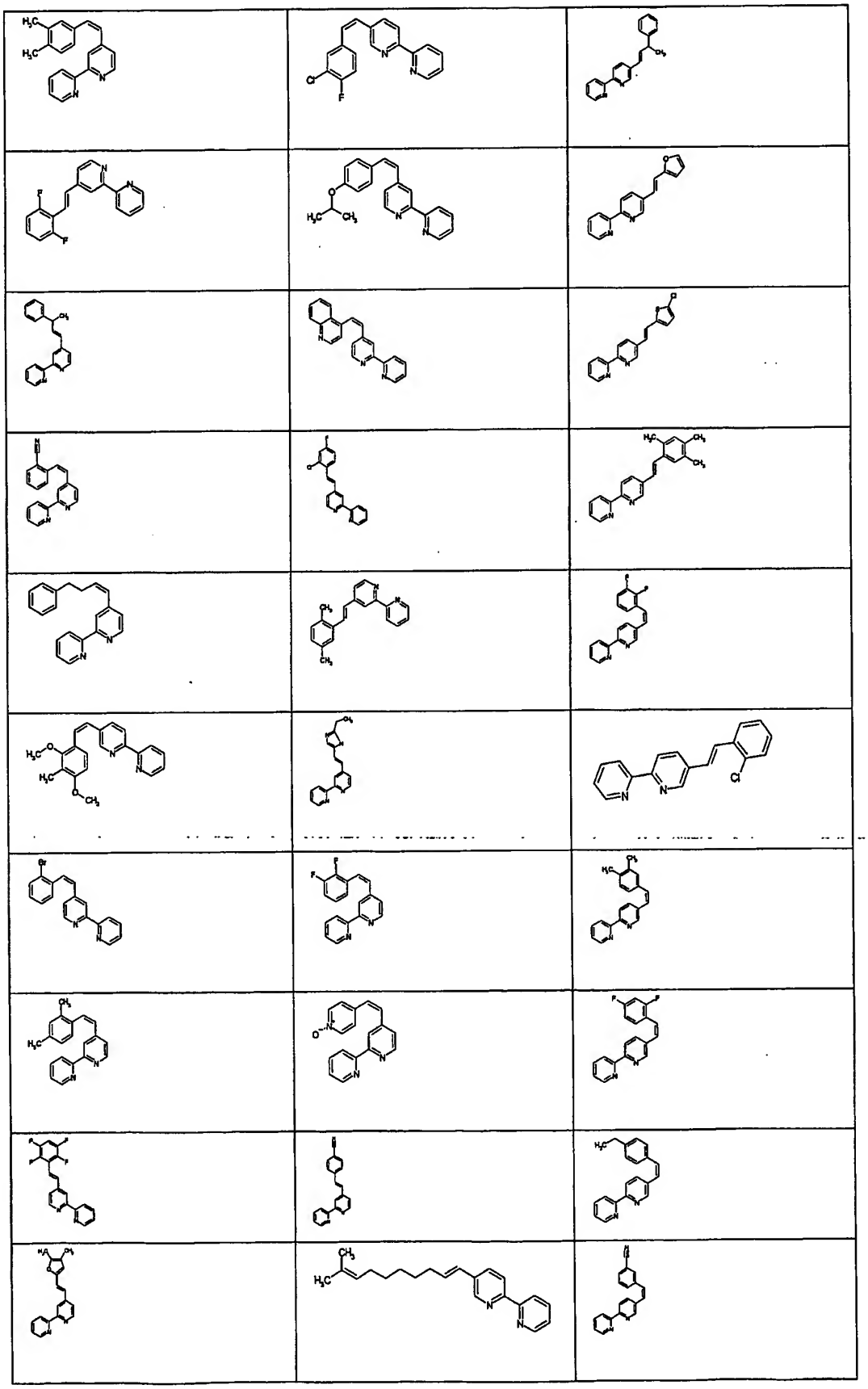
5-(2-Phenyl-1-ethenyll)-2,2´-bipyridine. Triphenyl phosphine (1.0 mmol, 0.262 g) was dissolved in dry benzene (7 ml). 4-(Bromomethyl)-2,2´-bipyridine (1.06 mmol, 0.265 g) was added, and the reaction solution was refluxed for 2 h. A white precipitate formed. The solvent was removed *in vacuo*, and DCM (7 ml) was added to the solid residue. Benzaldehyde (1.0 mmol, 0.102 ml) was added and thereafter aqueous NaOH (1.0 mmol, 0.25 ml, 4M). The reaction mixture was stirred at room temperature for 15 h. Addition of $MgSO_4$ to remove water was followed by filtration through a short silica column to yield a clear colourless solution. Purification by column chromatography (10 % EtOH/DCM) yielded the desired pure product. Yield: 83 %. ^1H NMR ($CDCl_3$, 300 MHz) (selected peaks): δ 6.84 (d, J = 12.1 Hz, 1H), 6.62 (d, J = 12.3 Hz, 1H).

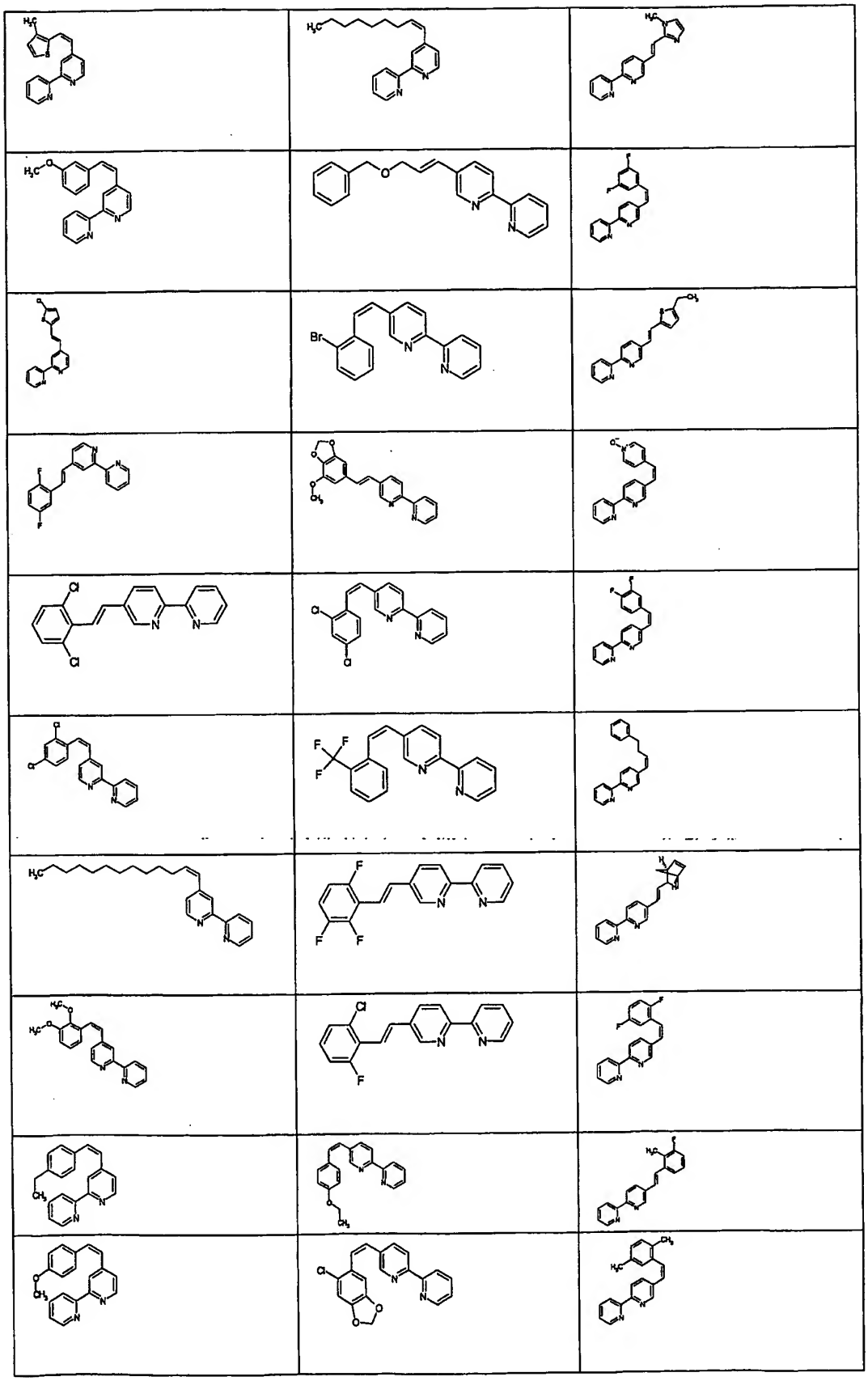
In analogous manners, bipyridyl alkenes have been synthesized according to Table 26-1.

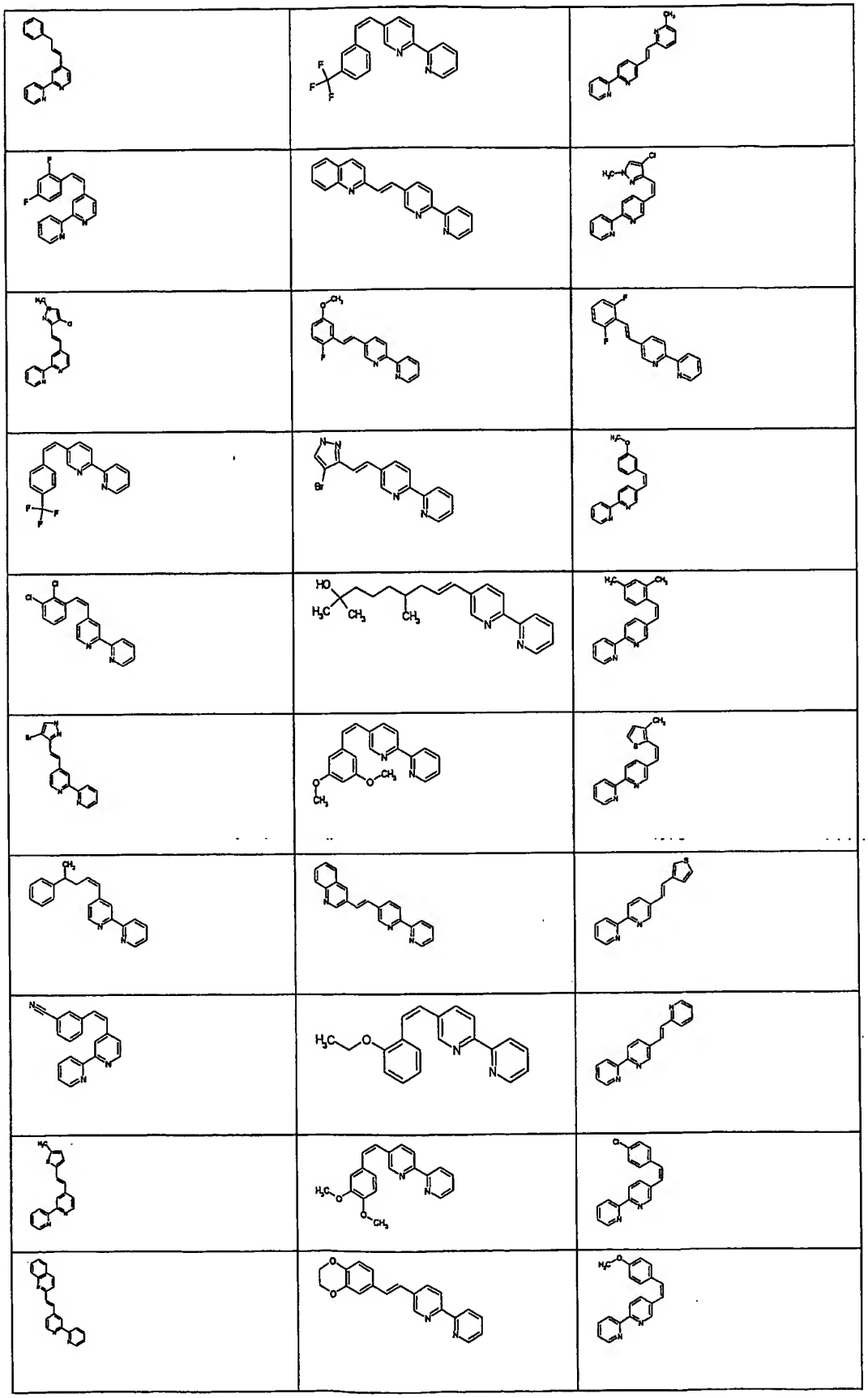
Table 26-1 Alkene derivatives prepared according to Example 26 in a typical "A"-type group.

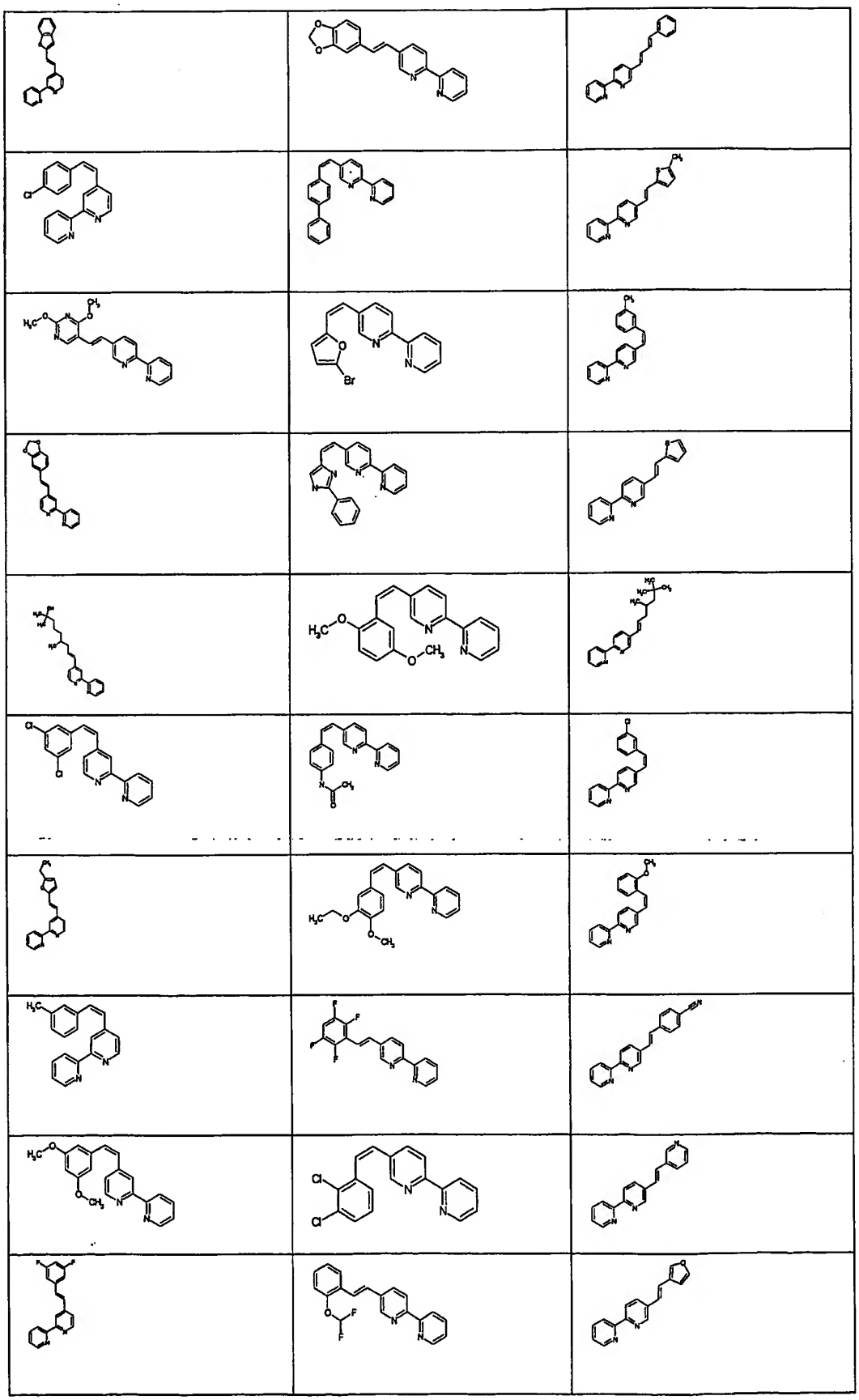
		

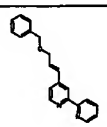
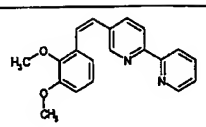
		
		
		
		
		
		
		
		
		



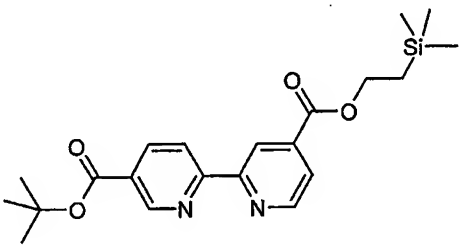






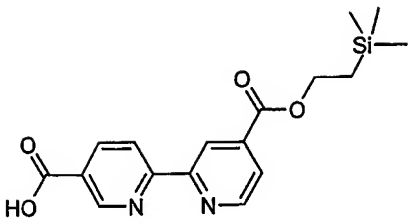
Example 27



4-(2-(trimethylsilyl)-ethylcarboxylate)-5'-(tert-butylcarboxylate)-2,2'-bipyridine. 6-Chloro-*tert*-butylnicotinate (12.7 mmol, 2.7g) was dissolved in dry *m*-xylene (150 ml) whereupon $Me_3SnSnMe_3$ (15.26 mmol, 5.0 g) was added together with $PdCl_2(PPh_3)_2$ (1.5 mmol, 1.0g). The reaction solution was heated to 130C under an N_2 atmosphere for 4h. 2-Chloro-(2-(trimethylsilyl)ethyl)-*iso*-nicotinate (15.26 mmol, 3.9g) was added and stirring was continued at 130C for 16h. The reaction mixture was allowed to cool to ambient temperature whereafter the solvent was evaporated *in vacuo*. The residue was taken up in DCM, and purified by column chromatography using DCM as the eluent. Yield: 62 %. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.29-9.25 (m, 1H), 9.05-9.02 (m, 1H), 8.88-8.84 (m, 1H), 8.56-8.51 (m, 1H), 8.42-8.36 (m, 1H), 7.96-7.92 (m, 1H), 4.53-4.48 (m, 2H), 1.65 (s, 9H), 1.24-1.18 (m, 2H), 0.12 (s, 9H).

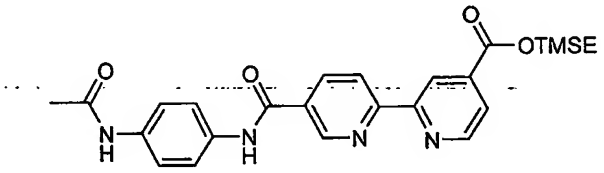
By the same method the corresponding 2,2'-bipyridyl-esters, -nitriles, -aldehydes, -protected amines and protected alcohols be synthesised in all possible combinations, and positions on the 2,2'-bipyridyl scaffold (*i.e.* AA'-, AC-groups).

Example 28



4-(2-(trimethylsilyl)-ethylcarboxylate)-5′-(carboxyacid)-2,2′-bipyridine. 4-(2-(Trimethylsilyl)-ethylcarboxylate)-5′-(*tert*-butylcarboxylate)-2,2′-bipyridine (2.5 mmol, 1.0 g) was dissolved in dry 1,4-dioxane (15 ml). Triethylamine (3.75 mmol, 0.523 ml) was added and TMSOTf (3.75 mmol, 0.679 ml) was added droppwise. Upon completion of addition the reaction solution was heated to 100C for 2h. Stirring was thereafter continued for 3h at 21C. Water was then carefully added and the formed precipitate was collected by filtration and the solid residue was washed several times with water and allowed to dry at room temperature for 24h. Yield: 71 %. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.21-9.18 (m, 1H), 8.94-8.90 (m, 1H), 8.87-8.83 (m, 1H), 8.54-8.52 (m, 1H), 8.45-8.42 (m, 1H), 7.93-7.90 (m, 1H), 4.49-4.43 (m, 2H), 1.18-1.15 (m, 2H), 0.08 (s, 9H).

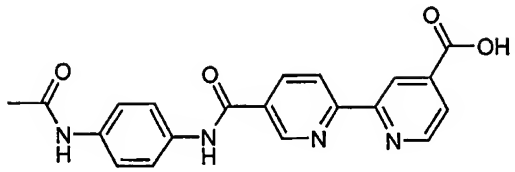
Example 29



4-(2-(trimethylsilyl)-ethylcarboxylate)-5′-(4-(acetanilido)carboxamide)-2,2′-bipyridine. 4-(2-(Trimethylsilyl)-ethylcarboxylate)-5′-(carboxyacid)-2,2′-bipyridine (1.45 mmol, 0.5g) was dissolved in DCM/DMF (5 ml / 5 ml). HBTU (1.74 mmol, 0.66g) was added and the mixture was stirred for 2h at room temperature. 4-Aminoacetanilide (1.74 mmol, 0.26g) was added in one portion, and stirring was continued at room temperature for another 16h. Water was added, and the reaction mixture was extracted with DCM. The combined organic phases were washed once with water and finally with brine prior to drying over $MgSO_4$, and evaporation *in vacuo*. Purification was made by column chromatography on neutral alumina using DCM/ ethanol (95:5) as eluent. Yield: 72 %. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.48 (s, 1H), 9.94 (s, 1H), 9.25-9.22 (m, 1H), 8.98-8.93 (m, 1H), 8.90-8.88 (m, 1H), 8.57-8.54 (m, 1H), 8.50-8.46 (m, 1H), 7.94-7.92 (m, 1H), 7.70 (d, J = 8.85 Hz,

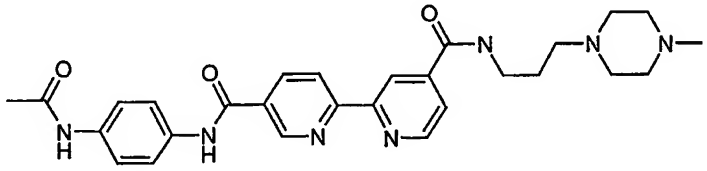
2H), 7.58 (d, *J* = 9.03 Hz, 2H), 4.51-4.46 (m, 2H), 2.04 (s, 3H), 1.19-1.14 (m, 2H), 0.09 (s, 9H).

Example 30



4-(carboxyacid)-5′-(4-(acetanilido)carboxamide)-2,2′-bipyridine. 4-(2-(Trimethylsilyl)-ethylcarboxylate)-5′-(4-(acetanilido)-carboxamide)-2,2′-bipyridine (0.042 mmol, 20 mg) was dissolved in THF (5 ml). To the solution was added TBAF (0.126 mmol, 126□l, 1M solution in THF). The solution was stirred at room temperature for 15h, whereupon it was made acidic with diluted HCl (1M solution) to pH = 3.5. The formed precipitate was collected by filtration and rinsed with several portions of water, and thereafter dried at room temperature for 24h. Yield: 67 %. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 13.80 (br. s, 1H), 10.47 (s, 1H), 9.92 (s, 1H), 9.24-9.23 (m, 1H), 8.95-8.84 (m, 2H), 8.57-8.54 (m, 1H), 8.48 (dd, *J* = 2.07, 7.95 Hz, 1H), 7.93 (dd, *J* = 1.68, 7.80 Hz, 1H), 7.70 (d, *J* = 8.46 Hz, 2H), 7.57 (d, *J* = 9.03 Hz, 2H), 2.04 (s, 3H).

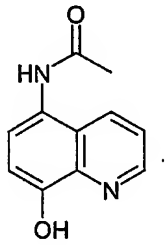
Example 31



4-(3′′-(N-methylpiperazine)-propyl)carboxamide)-5′-(4-(acetanilido)carboxamide)-2,2′-bipyridine. 4-(Carboxyacid)-5′-(4-(acetanilido)carboxamide)-2,2′-bipyridine (0.04 mmol, 15 mg) was dissolved in DCM/DMF (1:1, 5 ml), whereupon HBTU (0.05 mmol , 189 mg) was added in one portion. A few drops of triethylamine was added and the resulting mixture

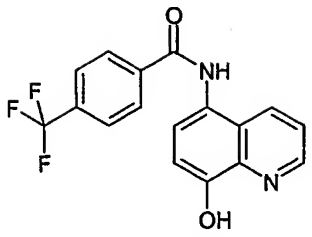
was stirred at room temperature for 3 h. 3-(*N*`-Methylpiperazine)-propyl amine (0.06 mmol , 9.4 mg) was added, and the reaction solution was stirred at room temperature over night. Water was added and the the organic layer was separated. The aqueous phase was extracted with DCM. The combined organic layers were washed with water, brine and finally sat. $CaCl_2$. Drying over $MgSO_4$, and evaporation *in vacuo* yielded a yellow viscous oil. Purification was made by column chromatography on neutral alumina using DCM/ ethanol (95:5) as eluent. Yield: 63 %. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.38 (s, 1H), 9.81 (s, 1H), 9.21-9.19 (m, 1H), 8.91-8.82 (m, 2H), 8.58-8.55 (m, 1H), 8.46 (dd, J = 1.97, 7.89 Hz, 1H), 7.90 (dd, J = 1.73, 7.81 Hz, 1H), 7.69 (d, J = 8.51 Hz, 2H), 7.53 (d, J = 9.10 Hz, 2H), 3.45-3.30 (m, 2H), 2.60-2.30 (m, 10H), 2.18 (s, 3H), 2.04 (s, 3H), 1.18-1.16 (m, 2H).

Example 32



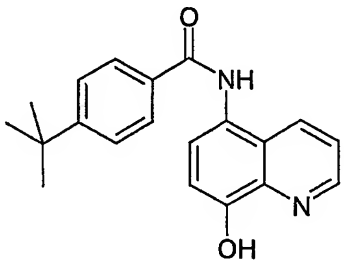
N-(8-Hydroxy-quinolin-5-yl)-acetamide. 5-Amino-8-hydroxyquinoline (1 mmol, 0.233g) was stirred in ether at ambient temperature before acetic anhydride (10 mmol, 1ml), followed by sodium acetate (10 mmol, 1.36g) was added. The resulting mixture was heated to 40 °C for 16 h before being diluted with ether (100ml) poured onto a saturated solution of ammonium chloride (50 ml). The organics were separated and washed with sodium bicarbonate (50 ml), water (3 x 50 ml), brine (50 ml), dried over sodium sulphate and concentrated *in vacuo*. Purification by column chromatography (30 % EtOAc/petrol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.11 (dd, J = 8.9, 1.7 Hz, 1H), 9.4 (dd, J = 4.0, 1.5 Hz, 1H), 8.46 (d, J = 8.4 Hz, 1H), 7.71 (dd, J = 8.8, 4.1 Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H), 2.56 (s, 3H).

Example 33



N-(8-Hydroxy-quinolin-5-yl)-4-trifluoromethyl-benzamide. 5-Amino-8-hydroxyquinoline (0.15mmol, 25 mg) was dissolved in dry dichloromethane (5 ml) before the sequential addition of dimethyl formamide (0.2 ml), *N,N,*-dimethylaminopyridine (1 crystal), PS-carbodiimide (750mg) and 1-hydroxybenzotriazole monohydrate (0.6mmol, 81 mg). The suspension was stirred for 72 h before the solids were removed by filtration and the resulting filtrate diluted with dichloromethane (20 ml), washed with sodium bicarbonate (2 x 20 ml), brine (20 ml), dried over sodium sulphate and concentrated *in vacuo*. The residue was then taken up in dichloromethane (50 ml), with methanol (10 ml) and water (1 ml). To this mixture was added lithium hydroxide (30mmol, 720 mg). The suspension was stirred for 16 h before the solids were removed by filtration and the resulting filtrate washed with sodium bicarbonate (20 ml), water (20 ml) and brine (20 ml), dried over sodium sulphate and purified by direct filtration through a plug of alumina. The alumina was washed with dichloromethane (100 ml) before the product was eluted with ethyl acetate. The volatiles were removed *in vacuo*. GC-MS: m/z = 332 (= $M^{+\cdot}$).

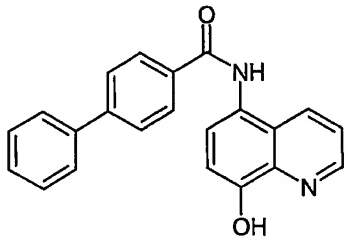
Example 34



4-tert-Butyl-N-(8-hydroxy-quinolin-5-yl)-benzamide. To a suspension of 5-amino-8.hydroxyquinoline dihydrochloride (1.0 mmol, 0.23 g) and dimethylaminopyridine (3 crystals) in dichloromethane (10 ml) at ambient temperature was added 4-*tert*-butylbenzoyl chloride (3.0 mmol, 0.59 ml). Stirring continued for 10 min before triethylamine (10mmol, 2.8 ml) was added in one portion. The solution was allowed to stir overnight before all volatiles were removed *in vacuo* and the residue purified directly by

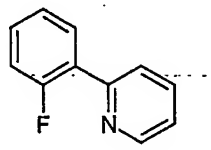
column chromatography (10 % EtOAc / hexane). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.13 (dd, J = 9.0, 1.7 Hz, 1H), 8.99 (dd, J = 4.1, 1.7 Hz, 1H), 8.52 (d, J = 8.5 Hz, 1H), 8.27 (dt, J = 8.5, 1.7 Hz, 2H), 7.69 (m, 2H), 7.61 (m, 2H), 1.43 (s, 9H).

In similar fashion the following compound was made:



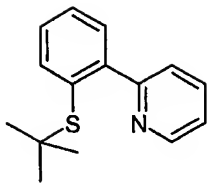
^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.14 (dd, J = 8.9, 1.5 Hz, 1H), 9.03 (dd, J = 4.0, 1.4 Hz, 1H), 8.57 (d, J = 8.5 Hz, 1H), 8.43 (app d, J = 8.1 Hz, 2H), 7.84 (app. d, J = 8.3 Hz, 2H), 7.70 (m, 4H), 7.50 (m, 4H).

Example 35



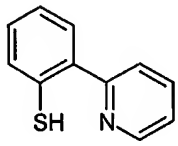
2-(2-Pyridyl)fluorobenzene: 2-Fluorophenylboronic acid (3.0 g, 21.4 mmol) was dissolved in DME (40 ml). 2-Bromopyridine (1.64 ml, 17.2 mmol) was added followed by 2M K_2CO_3 (20 ml). The mixture was degassed by bubbling nitrogen gas through for 34 min. Bis-(triphenylphosphine)palladium chloride (1.2 g, 1.72 mmol) was added and the mixture was heated to 80°C over night. The mixture was cooled to room temperature and filtered through celite. Extraction with H_2O (200 ml) and EtOAc (200 ml), drying the organic phase over $MgSO_4$, filter and evaporation gave the crude product. Purification by column chromatography (SiO_2, DCM: 10 % NH_4OH in MeOH 10:0.05). Yield: 2.4 g (80 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.18 (dt, J = 8.1, 1.13 Hz, 1H), 7.28 (m, 2H), 7.40 (m, 1H), 7.79 (m, 2H), 8.00 (dt, J = 7.72, 1.88 Hz, 1H), 8.75 (dt, J = 4.52, 1.32 Hz, 1H). LC-MS: m/z = 174 (=M+1)

Example 36



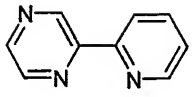
S-tert-Butyl-2-(2-pyridyl)thiophenol: DMF (10 ml) was degassed for 1hour and 10 minutes. Sodium hydride (60 % dispersion in mineral oil) (231 mg, 5.77 mmol) and 2-methyl-2-propanethiol (715 µl, 5.77 mmol) was added. The mixture was stirred for 7 minutes at room temperature. 2-(2-Pyridyl)fluorobenzene (500 mg, 2.89 mmol) was added, and the mixture was heated to 120 °C for 3 days. The mixture was cooled to room temperature. H_2O (50 ml) was added and the mixture was extracted with EtOAc (70 ml). The organic phase was washed with H_2O (50 ml), dried over $MgSO_4$, filtered and evaporated. Yield: ~100 %, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.04 (s, 9H), 7.25 (m, 1H), 7.39 (dt, J = 7.54, 1.51 Hz, 1H), 7.49 (dt, J = 7.54, 1.51 Hz, 1H), 7.69 (m, 4H), 8.70 (m, 1H). LC-MS: m/z = 244 (=M+1)

Example 37



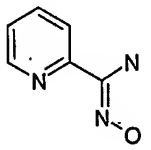
2-(2-Pyridyl)thiophenol: S-*tert*-Butyl-2-(2-pyridyl)thiophenol (200 mg, 0.82 mmol) was dissolved in 37 % HCl (4 ml) and the mixture was heated to 110 °C over night. The mixture was cooled to room temperature. H_2O (10 ml) was added and the mixture was extracted with EtOAc (20 ml). pH of the aqueous phase was adjusted to 7 and the mixture was extracted with EtOAc (50 ml). The organic phase was dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography (SiO2, EtOAc:Heptane 1:1). Yield: 66.2 mg (43 %), ^{1}H NMR (CDCl3, 300 MHz) δ 7.30 (m, 3H), 7.52 (m, 1H), 7.62 (m, 1H), 7.80 (m, 2H), 8.74 (m, 1H).

Example 38



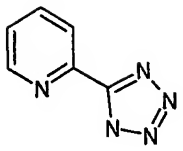
2-(2-Pyridyl)pyrazine. 2-Chloropyrazine (100 mg, 0.87 mmol) was dissolved in m-xylen (2 ml), 2-tri-*n*-butylstannylpyridin (354 mg, 0.96 mmol) was added followed by bis-(triphenylphosphine)palladium chloride (1.2 mg, 0.0017 mmol). The mixture was heated to 130 °C over night under nitrogen. The mixture was allowed to cool to room temperature. The crude mixture was purified by column chromatography (SiO2; EtOAc:Heptane 1:1). The product was dissolved in EtOAc (25 ml) and washed with aqueous HCl (pH ~ 3) (2 x 30 ml). The aqueous phase was adjusted to pH 8 with $NaHCO_3$ and extracted with EtOAc (2 x 20 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated. Yield: 53 mg (38 %). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.39 (m, 1H), 7.87 (dt, J = 7.91, 1.69 Hz, 1H),8.38 (m, 1H),8.62 (m, 2H),8.74 (m, 1H), 9.66 (s, 1H).

Example 39



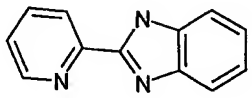
N-Hydroxy-pyridine-2-carboxamidine. Sodium (0.53 g 23 mmol) was dissolved in MeOH (15 ml), hydroxylamine hydrochloride (1.53 g 22 mmol) dissolved in MeOH (15 ml) was added, and stirred in ice bath for 1 hour. After filtration the solution was added 2-cyanopyridin (1.93 ml, 20mmol),and stirred at R.T. over night. The reaction mixture was reduced *in vacuo*. After cooling on ice the product precipitate. Filtered and washed with diethyl ether. Yield: 2.1 g (73 %). ^1H NMR ($CDCl_3$, 300 MHz) δ 5.75 (br s, 2H), 7.34 (ddd J = 1.32 Hz, J = 4.9 Hz, J = 7.53 Hz, 1H), 7.73 (dt, J = 1.88 Hz, J = 7.54 Hz, 1H), 8.94 (dt, J = 1.13 Hz, J = 7.92Hz, 1H), 8.18 (br s, 1H), 8.58 (ddd, J = 0.95 Hz, J = 1.88 Hz, J = 4.9 Hz, 1H).

Example 40



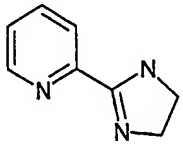
2-(5-Tetrazolyl)pyridyine. This chelator was prepared from the corresponding cyano-compound according to Example 15.

Example 41



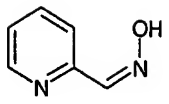
2-Pyridin-2-yl-1H-benzoimidazole. Picolinic acid (2.5 g, 20.3 mmol) was added THF (25 ml) and heated to reflux. Carbonyl diimidazole (3.6 g, 22.3 mmol) was added in portions, and the reaction was heated for 3 hours. After cooling to R.T., 1,2-phenylenediamine (2.2 g, 20.3 mmol) was added, and the reaction was stirred for 1 hour at R.T. Evaporated and dissolved in EtOAc, washed with water, dried and evaporated. The formed crystals were washed with diethyl ether and dried. Yield: 0.8 g.

Example 42



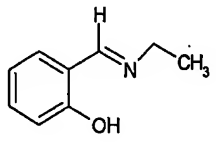
2-(4,5-Dihydro-1H-imidazol-2-yl)-pyridine. Picolinic acid (2.5 g, 20.3 mmol) was added THF (25 ml) and heated to reflux. Carbonyl diimidazole (3.6 g, 22.3 mmol) was added in portions, and the reaction was heated for 3 hours. After cooling to R.T., 1,2-ethylenediamine (1.4 ml, 20.3 mmol) was added, and a colorless precipitation was formed. The reaction was stirred for 1 hour at R.T. The solid was filtered off, washed with THF and dried. Yield: 1.3 g.

Example 43



Pyridine-2-carbaldehyde oxime. 2-Pyridylcarbaldehyde (0.5 g, 4.7 mmol) and hydroxylamine hydrochloride (0.65 g, 9.4 mmol) was dissolved in ethanol (30 ml) followed by pyridine (0.76 ml, 9.43 mmol). The reaction was heated to reflux for 2 hours and 20 minutes. After cooling, the reaction was evaporated, the crude redissolved in EtOAc, washed with water, dried and evaporated, to give a colourless crystalline solid. Yield: 0.46 g (80 %).

Example 44



2-Ethyliminomethyl-phenol. Salicylaldehyde (4.4 ml, 40.9 mmol) was dissolved in dry toluene (100 ml), and ethylamine (g) was bubbled through (3 x 5 minutes). Left at R.T. over night. Still starting material. The reaction was heated to 65 °C over night. Evaporated and distilled.

Example 45

Binding and activation of metal ions through interaction with an endogenous metal Ion-binding site in the MC-1 and the MC-4 receptor.

The present example describes the novel discovery that Zn(II) can bind to the wild type MC1 receptor and to the wild type MC-4 receptor with micromolar affinity. Furthermore it is observed that Zn(II) can act as an agonist on both of the two melanocortin receptors. The geometry of metal ion binding sites in general is well characterized from the crystal structure of metal-ion binding soluble proteins. The metal-ion binding site in the MC1 receptor is mapped by mutational substitution of potential metal-ion binding residues (histidine, cysteine, glutamate or aspartate residues) located in suitable positions in the

extracellular part of the receptor. In Fig. 1 these potential metal ion binding residues, which can be reached by extracellular acting ligands, are marked with grey.

Methods

The human MC-4 receptor cDNA was cloned by PCR from brain cDNA library whereas the mouse MC-1 receptor was kindly provided by Dr. R. Cone, U.S.A.. Both receptors were cloned into a eukaryotic expression vector and introduced into COS-7 cells by a standard calcium phosphate transfection method.

Binding assay: One day after transfection the cells were transferred and seeded in multi-well plates for assay. The number of cells plated per well was chosen so as to obtain 5 to 10% binding of the radioligand added. Two days after transfection the cells were assayed in competition binding assays using ^{125}I- NDP-α-MSH as a tracer. Radioligand was bound in a buffer composed of 0.5 ml of 50 mM Hepes buffer, pH 7.4, supplemented with 1 mM $CaCl_2$, 5 mM $MgCl_2$, and 0.1 % BSA, and displaced in a dose dependent manner by unlabelled ligands. The assay was performed in duplicate for 3 hours at 25 °C and stopped by washing twice in the buffer. Cell associated, receptor bound radioligand was determined by the addition of lysis buffer (48% urea, 2% NP-40 in 3M acetic acid). The concentration of radioligand in the assay corresponds to a final concentration of approximately 20 pM. The metal-ion chelating compounds were added in a two-fold molar excess in order to ensure that no free metal-ion was present.

cAMP assay: Two days after transfection the cells were assayed for intracellular levels of basal and ligand-induced cyclic AMP. The assay employed is essentially as described in Solomon et al (Anal.Biochem. (1974) 58: 541). Labelled adenine (2 μCi, [^{3}H]adenine, Amersham TRK311) was added to cells seeded in 6-well culture dishes. The following day the cells were washed twice with HBS buffer [25 mM Hepes, 0.75 mM NaH_2PO_4, 140 mM NaCl (pH 7.2)] and incubated in buffer supplemented with 1 mM 3-isobutyl-1-methylxanthine (Sigma I-5879). Agonists were added and the cells were incubated for 30 min at 37 °C. The assay was terminated by placing the cells on ice and aspiration of the buffer followed by addition of ice-cold 5% trichloroacetic acid containing 0.1 mM unlabelled camp (Sigma A-9062) and ATP (Sigma A-9501). Cyclic AMP was then isolated by application of the supernatant to a 50W-X4 resin (BioRad) and subsequently an alumina resin (A-9003; Sigma) eluting the cyclic AMP with 0.1 M imidazole (Sigma I-0125). Determinations were done in duplicate.

Results and discussion

Competition binding studies were performed for both the MC-1 and MC-4 receptors in transiently transfected COS-7 cells using ^{125}I-NDP-α-MSH as radioligand. Zn(II) displaced the ^{125}I-NDP-α-MSH with an affinity of 16 μM in the MC-1 receptor and 12 μM in the MC-4 receptor. However, Zn(II) was only able to displace approximately 50 % of the maximally bound ^{125}I-NDP-α-MSH, whereas the peptide ligands α-MSH and NDP-α-MSH displaced the radioligand fully, i.e. down to approximately 5 % unspecific binding, as shown in Fig. 2A, Fig. 2B and Table 45-1.

Table 45-1: Competition binding using ^{125}I-NDP-α–MSH as a radioligand in transiently transfected COS-7 cell expressing MC1 and MC4.

	MC1 receptor			MC4 receptor		
	K_d			K_d		
	μM	SEM	n	nM	SEM	n
MSH	5.0 nM	± 0.4	6	7.2	± 0.1	6
NDP MSH	2.1 nM	± 0.1	7	4.6	± 1.0	7
Zn	16	± 3	3	12	± 4	3

The functional consequence of the Zn(II) binding was evaluated by analysis of cAMP accumulation in transiently transfected COS-7 cells. Zn(II) acted as a partial agonist both in the MC-1 receptor and in the MC-4 receptor. On the MC-1 receptor Zn(II) had an efficacy of approximately 50 % as compared to α-MSH, whereas on the MC-4 receptor Zn(II) showed only 20 % efficacy compared to α-MSH (Fig. 2C, 2D). The potency of Zn(II) was 13 and 16 μM (EC50 values) for the MC-1 and the MC-4, respectively, which corresponds to the affinity for Zn(II) determined in the competition binding experiments.

Since zinc ions are stored in intracellular vesicles and co-released upon stimulation with different neurotransmitters it is possible that the concentration of free Zn(II) in the synaptic cleft reaches a very high level (conceivably up to maximally 200μM). Thus, it is possible that physiological concentrations of Zn(II) may regulate the melanocortin receptor activity and thus be a physiological co-regulator of the signal transduction through the MC receptors. In order to understand the molecular determinants that are responsible for the metal ion induced receptor activation a number of potential metal-ion binding residues

were mutated in the MC-1 receptor. Acidic residues, cysteines and histidines located either in the extracellular part of the transmembrane segments, in the extracellular loops or in the N-terminal were substituted with a residue without metal ion coordinating properties, either an alanine or another more conservative substitution. The observed potencies determined from the cAMP dose-response curves for both zinc and α-MSH in each of the mutant forms of the MC-1 receptor are listed in Table 45-2. Most of the mutations affected neither the zinc induced stimulation nor the α-MSH induced stimulation. In accordance with previous observations, substitution of the aspartic acid Asp^{121} located at the extracellular end of TM III (III:05 in the generic numbering system for 7TM receptors) both to an alanine and to an aspargine residue decreased the potency of the endogenous agonist α–MSH more than 100 fold (Gantz 1997and 2000). However, In the cellular system employed in this study, such decreases in potency would result in a complete elimination of the α-MSH induced signal. In this construct also Zn(II) did not induce agonism. However no firm conclusion can be made since it is not known whether the receptor is non-functional or whether the Asp^{121} is indispensable for both the endogenous peptide agonist and the Zn(II) induced agonism. Substitution of a histidine in TM VI:17, which is located rather "deep" in TMVI with an alanine likewise destroyed both the α-MSH and the Zn(II) induced agonism. Two other residues Cys^{265} and Cys^{273} located in the extracellular loop 3 were unable to be stimulated both by the α-MSH and by the Zn^{2+}. It is likely that these two cysteine residues in the loop between TM VI and TM VII in fact form a disulfide bridge, which is important for the overall structure of the receptor. However, substitution of the third cysteine Cys^{271} in the loop between TM VI and TM VII had only minimal effect on the α-MSH activation of the receptor but it completely eliminated the Zn(II) induced agonism (see Figure 3). Thus, this residue is of crucial importance for the zinc induced stimualtion of the MC receptor.

The affinity and potency of the metal ion interaction with the receptor indicate that the metal-ion binding site is composed of at least two metal ion coordinating residues. Thus, it is assumed that there are other residues involved in the metal-ion binding in addition to the positively identified Cys residue in extracellular loop 3. It is very likely that the metal ion is coordinated between the Cys and the Asp at the extracellular end of TMIII (TMIII:05), since this residue is located close in space and since the natural agonist, α-MSH also binds to this residue. The inner face of TM-III is generally accepted as being a major site for interactions of agonists in 7TM receptors in general. Such an interaction would imply a stabilization of TMIII relative to TMVI and VII, which previously been described by the inventors as being the main molecular determinant for the activation of 7TM receptors.

Table 45-2: cAMP accumulation measured in COS-7 cells transiently transfected with MC1 and
MC1 receptor mutations, where potential metal ion binding residues are substituted. The potency (EC50 value) obtained from dose-response curve of Zn(II) and α-MSH are listed.

			Zn(II)		α–MSH	
Construct	position	Sek	EC_{50} (μM)	N	EC_{50} (μM)	n
mMC1 –wt			11	5	90	5
Glu30Ala	n-term	+	16	5	87	5
Cys33Ala	n-term	+	31	3	117	3
Glu92Ala	II:20	+	NS	2	NS	
Glu92Gln	II:20		70	3	150	3
Glu100Ala	ecl1	+	NS	2	NS	2
Glu100Gln	ecl1		26	3	560	3
Asp115Ala	III:01	+	18	3	400	3
Asp115Asn	III:01	+	19	3	545	3
Asp119Ala	III:05	+	NS	2	NS	
Asp119Asn	III:05	+	NS	4	NS	4
Cys123Ser	III:09	+	12	3	327	3
His183Ala	V:02	+	14	3	37	3
Cys189Ala	V:08	?	15	4	198	4
His258Ala	VI:19	-	NS	3	NS	3
Cys265Ala	ecl3	?	NS	3	NS	3
His268Ala	ecl3		23	2	102	2
Cys271Ala	ecl3	+	NS	5	356	5
Cys273Ala	ecl3	+	NS	2	NS	2

The mutational analysis was performed in the MC-1 receptor. However, the residues identified to constitute the activating metal-ion site in the MC1 receptor are conserved in all the other MC receptors as shown in Figure 4. Thus it is expected that the agonistic effect of Zn(II) observed in the MC4 receptor is also a results of metal ion binding in this site. Importantly, since these residues are conserved among all the MC receptors it is expected that Zn(II) will function as an agonist in all the five MC receptors.

Example 46

Zn(II) mediated potentiation of the endogenous agonist α-MSH activation in the MC-1 and the MC-4 receptor

The present example describes the discovery that metal ions not only function as agonists as described in example No. 45, but that the metal ions also are able to modulate the α-MSH function as it increases both the potency and the efficacy of the natural, endogenous agonits, α-MSH.

Methods

See Example 45

Results and discussion

According to basic pharmacological theory a partial agonist should behave as an antagonists as it dose-dependently should bring the cAMP turnover down to its own maximal stimulatory level when occupying the receptor. However, the observed Zn(II) mediated inhibition of the agonist induced cAMP accumulation was in fact biphasic. As shown in Figure 5A, in the MC-1 receptor Zn(II) concentration from 1 to 10 micromolar inhibited the α-MSH induced cAMP stimulation with approximately 40 %. But, at higher concentrations Zn(II) induced an increase in the cAMP accumulation to approximately 60 % percent above the maximally achievable α-MSH response (Fig. 5A). In the MC-4 receptor a similar biphasic pattern was observed, though the inhibitory component was more pronounced as Zn(II) in concentrations from 1 to 10 micromolar inhibited NPD-α-MSH induced cAMP production with approximately 65 % (see Figure 5B). At higher concentrations from 10 to 100 micromolar Zn(II) the inhibitory effect of the metal ion apparently disappeared as the cAMP accumulation returned up the level observed with NPD-α-MSH alone (see Figure 5B). This experiment indicates that Zn(II) not only behaves as a partial agonist on MC receptors (see example 45), but apparently also can potentiate the ability of the peptide agonists, α-MSH and the peptide analog NDP-α-MSH, to activate the receptor. This was directly studied by performing dose-response experiments with α-MSH / NDP-α-MSH in the presence and absence of Zn(II). It was found that addition of a constant concentration of Zn(II) (10^{-4}M) shifted the dose-response curve for agonists to the left both in the MC-1 and the MC-4 receptors, indicating that Zn(II) acts as an enhancer or potentiator of α-MSH (Figure 5C and 5D). In the MC1 receptor the α-MSH potency without Zn(II) was 116 μM and in the presence of zinc (10^{-4} M) it was increased approx. six-fold to 20 μM (Figure 5C). Whereas, in the MC-4 receptor zinc ions (10^{-4} M) induced a more limited two-fold increas in the potency of the dose response curve from 1.1 nM to 0.6 nM (Figure 5D). However, such potentiating effects can be very useful in the in vivo setting.

As suggested from Figure 5 the efficacy of α-MSH was affected by the presence of Zn(II). According to the partial agonism of Zn(II) the "basal level" of the α-MSH dose response curve in presence of 10^{-4} M of Zn(II) was approximately 50% of the maximal α-MSH induced stimulation. Similarly, the maximal stimulatory effect of α-MSH was increased to 160 percent the efficacy. No effect was observed in untransfected cells.

It is possible that a Zn(II) binding to the α-MSH peptide ligand as such concomitantly with its binding to the receptor may explain the modulating function of Zn(II) on both the potency and the efficacy of α-MSH. The peptide sequence of the α-MSH is Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val and accordingly the Zn(II) may bind with high affinity to the Histidine and / or the Glutamate residue a sequence, which previously has been demonstrated to be very importnant for the function of the peptide. Especially the –His-Phe-Arg-Trp- sequence is very important for the function of MSH peptides. As indicated in Fig. 6A, Zn(II) may bind in between Asp^{119} and Cys^{271} when the metal-ion alone or in complex with metal-ion chelators act as a partial agonist as presented in the present invention – in accordance with the fact that the inner ace of TM-III is an interaction site for agonists in general in 7TM receptors. However, as shown in Fig. 6B in the presence of an MSH peptide ligand, the Arg of the agonist ligand is supposed to interact with Asp^{119} in TM-III of the receptor and the potentiating action of the metal-ion described in the present invention could therefor be mediated through the binding of the metal-ion in between Cys^{271} of the receptor andthe His residue of the core tetra-peptide sequence of the ligand.

Example 47

Metal ion chelators and metal ion chelates can be used as agonists in the MC-1 and the MC-4 receptor

In examples 45 and 46, the discovery that Zn(II) can act as both an agonist and as a potentiator on the MC receptors was described. Both agonists but also antagonists of the MC receptors would be very useful compounds to have as drugs for obesity, erectile dysfunction etc.; however metal-ions as such are not very suited as drugs since they are toxic at the concentrations required for receptor binding and activation. In the present example, the discovery that not only metal ions but also the metal-ion bound in a small organic chelator can bind to the metal-ion binding sites of the MC receptors, and can act as agonists. Importantly, the metal-ion chelates, i.e. the complex between the metal-ion and the chelator in some cases showed both higher potency and higher efficacy clearly demonstrating that metal-ion chelates can be useful compounds as MC receptor

modulators and potential drugs. Importantly, it should be noted that the binding of the metal-ion in a chelate also create a greater degree of specificity especially when the chelator is chemically modified and optimized for interaction with the intended receptor.

Methods

See Example 45

Results and discussion

In the melanocotin receptors a couple of basic chelates, Zn-phenanthroline and Zn-bipyridine, were both found to bind with an affinity similar to that of the free Zn(II) i.e. at 15 μM and 12 μM, respectively (Table 47-1). In contrast to the free metal ion the chelates inhibited the radioligand binding more efficiently i.e. all the way down to the unspecific level (data not shown) like the natural ligand does. Six analogs of phenanthroline and three analogs of bipyridine (structures shown in Fig. 7) were tested for their respective binding affinities to both the MC-1 and the MC-4 receptors.

Table 47-1: Competition binding using ^{125}I-NDP-α–MSH as a Radioligand in transiently transfected COS-7 cell expressing MC1 and MC4.

	MC1 receptor			MC4 receptor		
	K_d			K_d		
	μM	SEM	n	nM	SEM	n
Zn	16	± 3	3	12	± 4	3
Zn(Dip)	22	± 7	3	22	± 7	3
Zn(Phe)	24	± 4	3	33	± 5	3
Zn(131)	1.4	± 0.2	3	1.2	± 0.1	3
Zn(132)	7.3	± 0.3	3	5.3	± 1.0	3
Zn(133)	8.9	± 2.5	3	10.0	± 3	3
Zn(134)	1.2	± 0.1	3	1.4	± 0.2	3
Zn(135)	20.0	± 7	3	12.0	± 1	3
Zn(136)	9.7	± 1.7	3	6.9	± 0.4	3
Zn(238)	14.0	± 1	3	18.0	± 5	3
Zn(315)	7.0	± 3.1	3	9.5	± 2.5	3
Zn(373)	4.5	± 1.1	3	9.9	± 4.1	3

Almost all of the Zn-phenanthroline analog tested showed an increase in affinity compared to Zn^{2+}-phenanthroline as such – i.e. two to 20 fold increase in affinity. Similarly, most of the bipyridine analogs in complex with Zn(II) had an increased affinity for the MC-1 and MC-4 receptor compared to the basic Zn-bipyridine chelate. Importantly, the two melanocortin receptors had a rather similar pharmacological profile for the metal ion chelator complexes when tested in competition binding analysis. An increase in the affinity on the MC-1 receptors was closely correlated to an increase in affinity on the MC-4 receptor, as shown in Figure 8. This observation supports the notion, that the metal-ion site is similarly located in the receptor structure of the two receptors, the MC-1 and MC-4. As noted previously the residues identified to be involved in the metal-ion binding are conserved among all the MC receptors.

Table 47-2: cAMP accumulation in transiently transfected COS7 cells with MC1 and MC4. The efficacies expressed as percent of maximum Zn(II) induced stimulation for the different Zn(II) chelates are listed. For some of the chelates the potency(EC_{50}) are measured.

	MC1 receptor					MC4 receptor				
HM compounds	Efficacy (% of max Zn^{2+})	sem	EC_{50} (μM)	sem	N	Efficacy (% of max Zn^{2+})	sem	EC_{50} (μM)	sem	N
Zn	100	± 20	11	± 4	7	100	8	13	4.8	
ZnDip	30	± 10	82	± 13	3	NS				2
ZnPhe	43	± 15	12	± 3	3	NS				2
Zn(131)	NS				2	NS				2
Zn(132)	167	± 40	8.1	± 1.5	4	NS				3
Zn(133)	NS				2	NS				2
Zn(134)	NS				2	NS				2
Zn(135)	NS				2	NS				2
Zn(136)	60	± 15			3	NS				2
Zn(238)	65	± 12			3	NS				2
Zn(315)	78	± 11			3	62	± 15	2.7	0.8	3
Zn(373)	87	± 19			3	53	± 12			3

Zn-phenanthroline and Zn-bipyridine as well as the analogues were tested in a functional, cAMP accumulation assay at a single, maximal concentration (10^{-4} M), i.e. tested for their ability to activate the receptors (Table 47-2). In contrast to the findings in the competition binding experiments, MC-1 and MC-4 exhibited very different pharmacological profile for the chelates when tested in the functional analysis. The MC-1 receptor was more prone to

become activated by the metal chelator complexes than the MC-4 receptor. This indicates that it will be possible to develop receptor selective metal-ion chelates.

The molecular structure of the chelator compounds was of crucial importance for the degree of activation they induced. The two basic chelator compounds Zn-phenanthroline and Zn-bipyridine (Figure 9A and Table 47-2) had a lower efficacy than the free zinc ion alone. However, when the chelates were further substituted it was possible to recover the activity. One of the compounds Zn-(5-chloro-1,10-phenanthroline) had a higher efficacy reaching 168 % of the Zn(II) induced efficacy and a 2 fold increased potency compared to free zinc (Figure 9A). Thus, this compound is nearly as efficacious as the natural agonist MSH, as it is a full agonist on the MC-1 receptor. Other Zn^{2+}-chelates, Zn-(5-amino-1,10-phenanthrolin) and three different bipyridine derivates activated the MC-1 receptor with an efficacy slightly lower than Zn(II), but clearly better than the chelate without substitutions. On the MC-4 receptor, on which Zn(II) is only a 20 percent partial agonist, none of the compounds had a higher efficacy than the free zinc. However, two of the bipyridine compounds, e.g. ZN(315) stimulated the receptor with a higher potency than the free zinc ion (Fig. 9B).

The compounds that bind to the melanocortin receptors but do not activate the receptors are antagonists as illustrated with one of the compounds Zn(134) on the MC-1 receptor (Figure 9).

Thus, even minor chemical modification of the structure of a metal-ion chelator complex, clearly altered its potency and affinity. Importantly, both more potent and more efficacious compounds were found in these mini-libraries of chelates – with one compound even being almost a full agonist. This demonstrates that metal-ion chelates or metal-ion chelators, which pick up a metal-ion in the organism, can be useful compounds to regulate melanocortin receptors activity also in the whole animal and in humans.

CLAIMS

1. A method for reducing overweight and/or for treating of and/or preventing overweight, obesity and/or complications thereto, the method comprising administering to an animal such as, e.g. a human and/or a domestic animal in need thereof an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor.

2. A method for treating and/or preventing diabetes mellitus type II, the method comprising administering to an animal such as, e.g. a human and/or a domestic animal in need thereof an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor.

3. A cosmetic method for reducing overweight, the method comprising administering to an animal such as, e.g., a human and/or a domestic animal in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor.

4. A method for reducing the fat tissue mass /lean mass body mass ratio in a human or domestic animal, the method comprising administering to a human or a domestic animal an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor.

5. A method for treating and/or preventing conditions involving the immune system, the method comprising administering to a human or a domestic animal an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor.

6. A method according to any of the preceding claims, wherein the chelate and/or chelator have agonistic activity against an MC receptor.

7. A method for treating and/or preventing anorexia and/or other appetite disorders, the method comprising administering to an animal such as, e.g., a human and/or a domestic animal in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor.

8. A method according to claim 7, wherein the chelate and/or chelator have antagonistic activity against an MC receptor.

9. A method for treating and/or preventing male or female sexual dysfunction such as, e.g. psychogenic sexual dysfunction of a mammal including a human, the method comprising administering to the mammal in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor.

10. A method for treating and/or preventing erectile dysfunction in a mammal including a human, the method comprising administering to the mammal in need thereof an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC receptor.

11. A method according to claim 9 or 10, wherein the chelate and/or chelator have agonistic activity against an MC receptor.

12. A method according to any of the preceding claims, wherein the MC receptor is selected from the group consiting of MC-1R, MC-2R, MC-3R, MC-4R, MC-5R including homo- and heterodimers, trimers and oligomers thereof.

13. A method according to any of the preceding claims, wherein the MC receptor is MC-1R, MC-3R or MC-4R.

14. A method according to any of the preceding claims, wherein the MC receptor is a mammalian MC receptor such as, e.g., a human MC receptor, a dog MC receptor, a cat MC receptor, a mouse MC receptor or a rat MC receptor.

15. A method for treating and/or preventing conditions involving the immune system, the method comprising administering to an animal such as, e.g. a human in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC-1 receptor.

16. A method for treating and/or preventing chronic and acute inflammation, the method comprising administering to an animal such as, e.g. a human in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC-1 receptor.

17. A cosmetic method for obtaining a suitable tan of the skin of an animal including a human, the method comprising administering to an animal such as, e.g. a human in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC-1 receptor.

18. A method according to claims 15-17, wherein the chelate and/or chelator have agonistic and/or antagonistic activity against an MC-1 receptor.

19. A method for treating and/or preventing steroidal disorders such as, e.g. Cushing's syndrome, the method comprising administering to an animal such as, e.g. a human in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC-2R.

20. A method according to claim 19, wherein the chelate and/or chelator have agonistic and/or antagonistic activity against an MC-2 receptor.

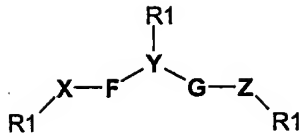
21. A method for treating and/or preventing perspiration disorders such as, e.g. sweat deficiency e.g. hypohidrosis, or excessive sweating e.g. diaphoresis or hyperhidrosis, the method comprising administering to an animal such as, e.g. a human in need thereof, an effective amount of a chelate and/or a chelator which is capable of binding or otherwise interacting with the natural metal-ion binding site in an MC-5 receptor.

22. A method according to claim 21, wherein the chelate and/or chelator have agonistic and/or antagonistic activity against an MC-5 receptor.

23. A method according to any of the preceding claims, wherein a chelator is administered together with a suffiecient amount of a suitable metal ion in the form of e.g. a metal salt, complex or covalent compound.

24. A method according to claims 1, 11-14, wherein the complications to overweight and/or obesity are selected from the group consisting of diabetes type II, hypertension, hypercholesterolaemia, hypertriglyceridaemia, cardiovascular diseases and/or arthritic diseases.

25. A method according to any of the preceding claims, wherein the chelate or chelator has the following general formula I



Formula I

wherein F is N, O, S, Se or P; and G is N, O, S, Se or P;

X, Y and Z, which are the same or different, are straight or branched C_1-C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} alkynyl, C_1-C_{12} cyclyl, aryl, C_1-C_{12} heteroalkyl, C_1-C_{12} heteroalkenyl, C_1-C_{12} heteroalkynyl, C_1-C_{12} heterocyclyl, heteroaryl;

R^1 may be present anywhere on the X, Y and/or Z moiety and it may be present on X, Y and/or Z up to as many times as possible, i.e. if X is $-CH_2-CH_2-$, then R^1 may be present on the first and/or second carbon atom one or several times; R1 may optionally be hydrogen;

X may together with Y and/or Z fuse to form a cyclic ring system;
Y may together with X and/or Z fuse to form a cyclic ring system;
X, Y and Z may together fuse to form a cyclic ring system;

R^1 corresponds to a structure –A-B-C, wherein the element A is a coupling or connecting moiety, B is a spacer moiety and C is a functional group; –B- may be substituted one or more times with a further C, which may be the same or different, and

A linked to be –A-B-C is selected from the group consisting of:

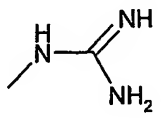
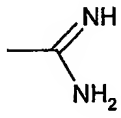
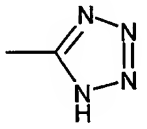
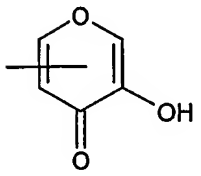
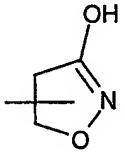
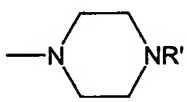
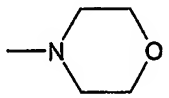
-O-, -S-, -NH-, -N=, -N<, $-CH_2-$, -C(=O)-, $-PO_3-$, $-PO_2NH-$, $-NHPO_2$, -NHP(O)<, -C≡C-, -CH=CH-, -SO-, $-SO_2-$, -COO-, -CONR"-, -NR'CO-, $-NR'SO_2-$, $-SO_2NR"-$, -CH(OH)-, -CR'(OH)-, -CR'(O-alk)-, -N-alk-, aryl, cycloalkyl, heteroaryl, heterocycloalkyl etc., and the term "Alk" includes straight or branched alkyl, straight or branched alkenyl and straight or branched alkynyl; R' is H or lower alk, i.e. C_1-C_6; R" is as defined below;

-B- is absent or selected from the group consisting of:

H, alkyl, straight or branched alkyl, alkenyl (straight or branched), alkynyl (straight or branched), aryl, cycloalkyl, heteroaryl, heterocycloalkyl, alkyloxyalkyl, alkylaminoalkyl,

-C is absent or selected from the group consisting of:

-H, -OH, -NR"R"', -CONR"R"', -COOR", -OCOR", -COR", -SO_2NR"R"', -SH, -S-S-alk, -NHCOR", -NR"COR"', $NHSO_2R$", -$NHCONH_2$, -NH-CN, -F, -Cl, -Br, -I; -SCF_3, -CF_3, -OCF_3, -SCH_3, -SR", -CN, -$N(CN)_2$,-NO_2, -OCH_3, -OR', -NH_2, -NHMe, -NHAlk, -NMe_2, -$NAlk_2$, -NMeAlk, -$N(Alk)_3^+$, heteroaryl, heterocycloalkyl

and R" and/or R"' has the same meaning as given for B above optionally substituted with one or more C;

in those cases where a compound has two or more R^1 in positions adjacent to each other the –A- and/or –B- elements from the two individual R^1 may form a cyclic ring system;

in those cases where B is absent R^1 is –A-C or –A and in those cases where C is absent R^1 is –A-B or –A;

in some cases, A may be absent and then $-R^1$ is –B-C or –C, and B may be substituted one or more times with C, which may be the same or different;

the total number of atoms (X+F+Y+G+Z) excluding hydrogen atoms is at the most 25;

the total number of heteroatoms in (X+F+Y+G+Z) is at the most 6; and

the size of a ring is at the most 14 atoms, preferably 5 or 6 atoms.

26. Use of a chelate and/or a chelator for the preparation of a pharmaceutical or cosmetic composition for reducing, treating and/or preventing i) overweight, obesity and/or complications thereto, ii) diabetes mellitus type II, iii) male or female sexual dysfunction iv)

erectile dysfunction, v) anorexia or other appetite disorders, vi) vi) chronic and acute inflammation and conditions involving the immune system, vii) tanning of the skin, viii) steroidal disorders or ix) perspiration disorders.

27. Use of a chelate and/or a chelator as a lead compound in a drug discovery process for identifying ligands that interact with an MC receptor.

28. Use according to claim 26 or 27, wherein the chelate or chelator is according to formula I in claim 25.

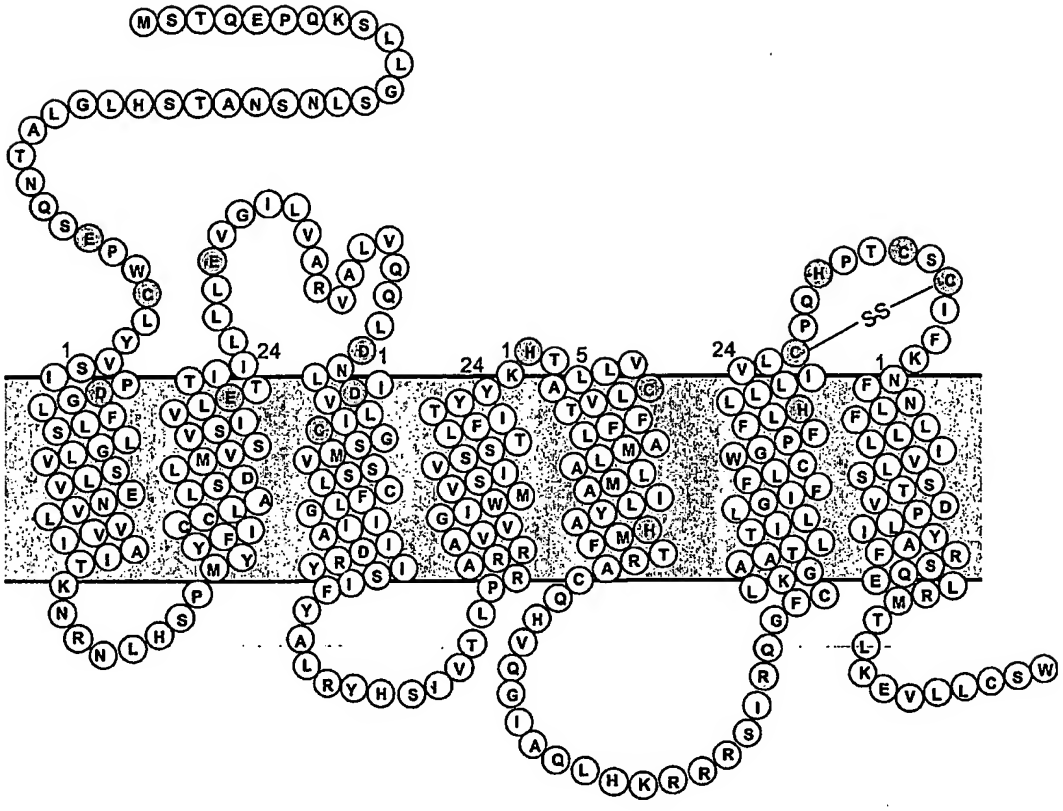


Fig. 1

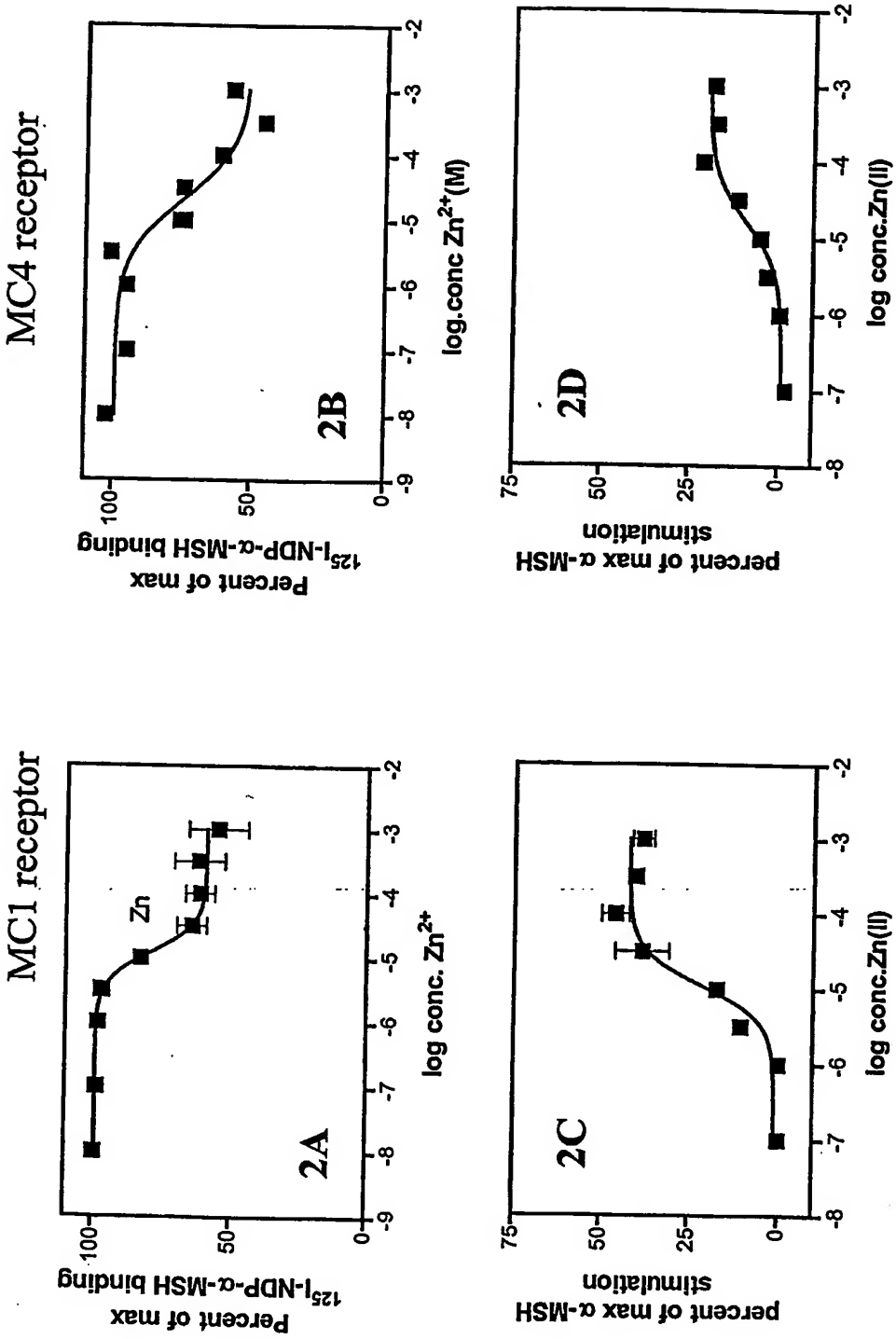

MC4 receptor
MC1 receptor
2A
2B
2C
2D
Zn
Percent of max 125I-NDP-α-MSH binding
log conc. Zn2+
log.conc Zn2+(M)
percent of max α-MSH stimulation
log conc.Zn(II)

Fig. 2

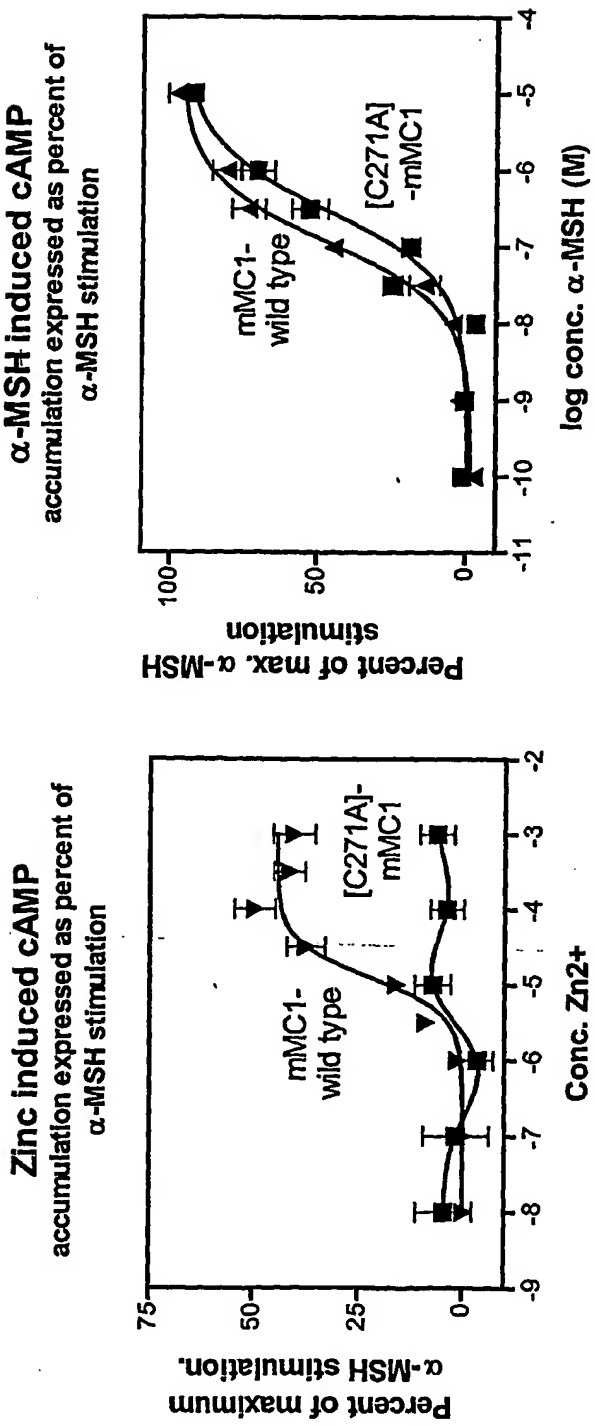

α-MSH induced cAMP
accumulation expressed as percent of
α-MSH stimulation
Percent of max. α-MSH
stimulation
100
50
0
-4
-5
-6
-7
-8
-9
-10
-11
log conc. α-MSH (M)
mMC1-
wild type
[C271A]
-mMC1
Zinc induced cAMP
accumulation expressed as percent of
α-MSH stimulation
Percent of maximum
α-MSH stimulation.
75
50
25
0
-2
-3
-4
-5
-6
-7
-8
-9
Conc. Zn2+
mMC1-
wild type
[C271A]-
mMC1

Fig. 3

4/10

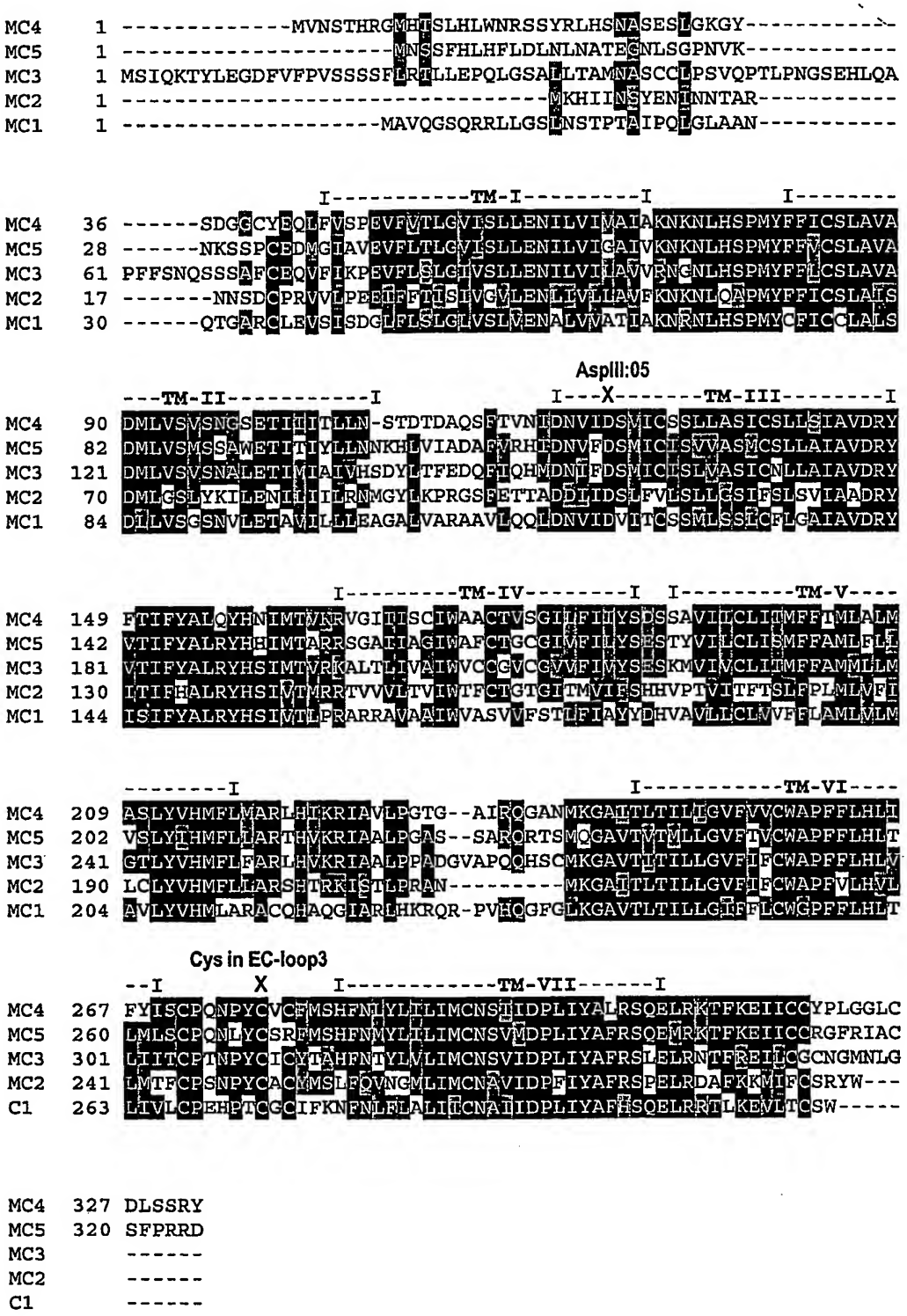

MC4 1 ----------MVNSTHRGMHISLHLWNRSSYRLHSNASESLGKGY----------
MC5 1 ----------MNSSFHLHFLDLNLNATEGNLSGPNVK----------
MC3 1 MSIQKTYLEGDFVFPVSSSSFLRTLLEPQLGSALLTAMNASCCLPSVQPTLPNGSEHLQA
MC2 1 ----------MKHIINSYENINNTAR----------
MC1 1 ----------MAVQGSQRRLLGSLNSTPTAIPQLGLAAN----------
I----------TM-I---------I I--------
MC4 36 ------SDGGCYEQLFVSPEVFVTLGVISLLENILVIMAIAKNKNLHSPMYFFICSLAVA
MC5 28 ------NKSSPCEDMGIAVEVFLTLGVISLLENILVIGAIVKNKNLHSPMYFFVCSLAVA
MC3 61 PFFSNQSSSAFCEQVFIKPEVFLSLGIVSLLENILVILAVVRNGNLHSPMYFFLCSLAVA
MC2 17 -------NNSDCPRVVLPEEIFFTISIVGVLENLIVLLAVFKNKNLQAPMYFFICSLAIS
MC1 30 ------QTGARCLEVSISDGLFLSLGLVSLVENALVVATIAKNRNLHSPMYCFICCLALS
AspIII:05
---TM-II----------I I---X--------TM-III--------I
MC4 90 DMLVSVSNGSETIIITLLN-STDTDAQSFTVNIDNVIDSVICSSLLASICSLLSIAVDRY
MC5 82 DMLVSMSSAWETITIYLLNNKHLVIADAFVRHIDNVFDSMICISVVASMCSLLAIAVDRY
MC3 121 DMLVSVSNALETIMIAIVHSDYLTFEDQFIQHMDNIFDSMICISLVASICNLLAIAVDRY
MC2 70 DMLGSLYKILENIIIILRNMGYLKPRGSFETTADDLIDSLFVLSLLGSIFSLSVIAADRY
MC1 84 DILVSGSNVLETAVILLLEAGALVARAAVLQQLDNVIDVITCSSMLSSICFLGAIAVDRY
I----------TM-IV---------I I----------TM-V----
MC4 149 FTIFYALQYHNIMTVKRVGIIISCIWAACTVSGILFIIYSDSSAVILCLITMFFTMLALM
MC5 142 VTIFYALRYHHIMTARRSGAIIAGIWAFCTGCGIVFILYSESTYVILCLISMFFAMLPLL
MC3 181 VTIFYALRYHSIMTVRKALTLIVAIWVCCGVCGVVFIMYSESKMVIVCLITMFFAMMLLM
MC2 130 LTIFHALRYHSIVTMRRTVVVLTVIWTFCTGTGITMVIFSHHVPTVITFTSLFPLMLVFI
MC1 144 ISIFYALRYHSIVTLPRARRAVAAIWVASVVFSTLFIAVYDHVAVLLCLVVFFLAMLMLM
--------I I----------TM-VI----
MC4 209 ASLYVHMFLMARLHIKRIAVLPGTG--AIRQGANMKGAITLTILIGVFVVCWAPFFLHLI
MC5 202 VSLYIHMFLLARTHVKRIAALPGAS--SARQRTSMQGAVTVTMLLGVFTVCWAPFFLHLT
MC3 241 GTLYVHMFLFARLHVKRIAALPPADGVAPQQHSCMKGAVTLTILLGVFIFCWAPFFLHLV
MC2 190 LCLYVHMFLLARSHTRKISTLPRAN---------MKGAITLTILLGVFIFCWAPFVLHVL
MC1 204 AVLYVHMLARACQHAQGIARLHKRQR-PVHQGFGLKGAVTLTILLGIFFLCWGPFFLHLT
Cys in EC-loop3
--I X I----------TM-VII------I
MC4 267 FYISCPQNPYCVCFMSHFNLYLILIMCNSTIDPLIYALRSQELRKTFKEIICCYPLGGLC
MC5 260 LMLSCPQNLYCSRFMSHFNMYLTLIMCNSVMDPLIYAFRSQEMRKTFKEIICCRGFRIAC
MC3 301 LIITCPTNPYCICYTAHFNTYLVLIMCNSVIDPLIYAFRSLELRNTFREILCGCNGMNLG
MC2 241 LMTFCPSNPYCACFMSLFQVNGMLIMCNAVIDPFIYAFRSPELRDAFKKMIFCSRYW---
C1 263 LIVLCPEHPTCGCIFKNFNLFLALIICNAIIDPLIYAFHSQELRRTLKEVLTCSW-----
MC4 327 DLSSRY
MC5 320 SFPRRD
MC3 ------
MC2 ------
C1 ------

Fig. 4

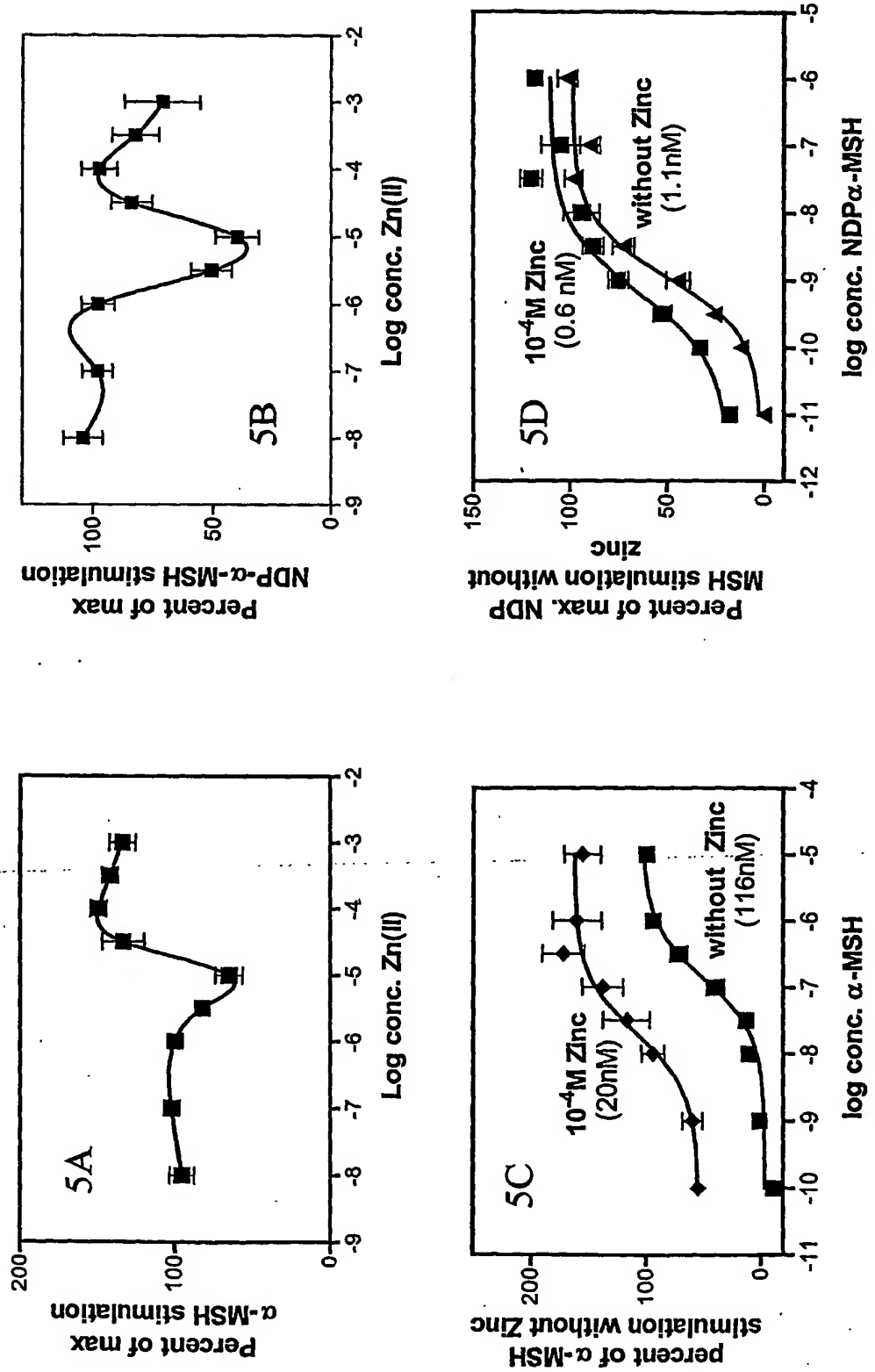

5A
Percent of max α-MSH stimulation
Log conc. Zn(II)
5B
Percent of max NDP-α-MSH stimulation
Log conc. Zn(II)
5C
percent of α-MSH stimulation without Zinc
log conc. α-MSH
10-4M Zinc (20nM)
without Zinc (116nM)
5D
Percent of max. NDP MSH stimulation without zinc
log conc. NDPα-MSH
10-4M Zinc (0.6 nM)
without Zinc (1.1nM)

Fig. 5

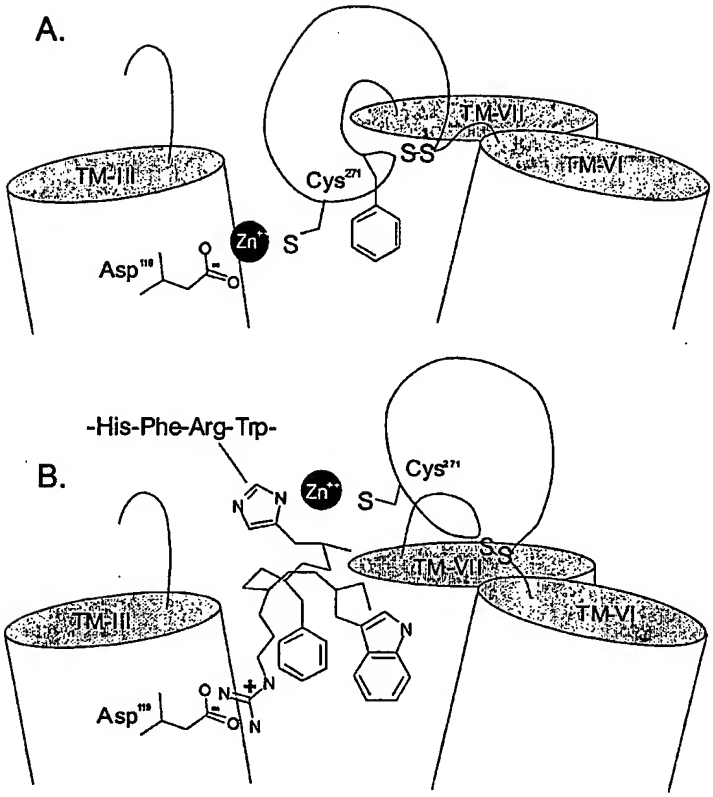

A.
TM-II
TM-VII
TM-VI
SS
Cys271
Zn++
S
Asp118
B.
-His-Phe-Arg-Trp-
Zn++
S
Cys271
SS
TM-VII
TM-II
TM-VI
Asp119

Fig. 6

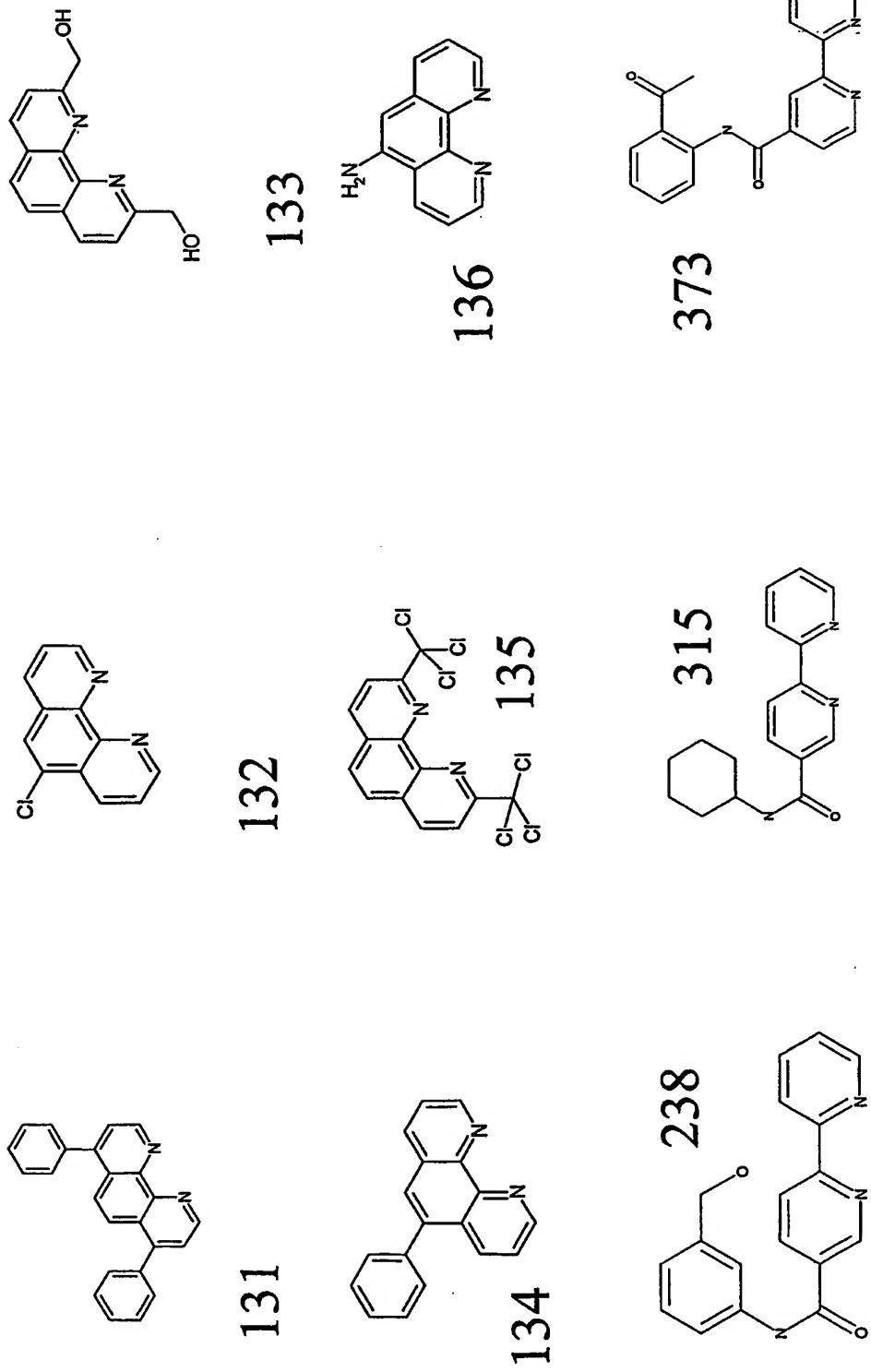


Fig. 7

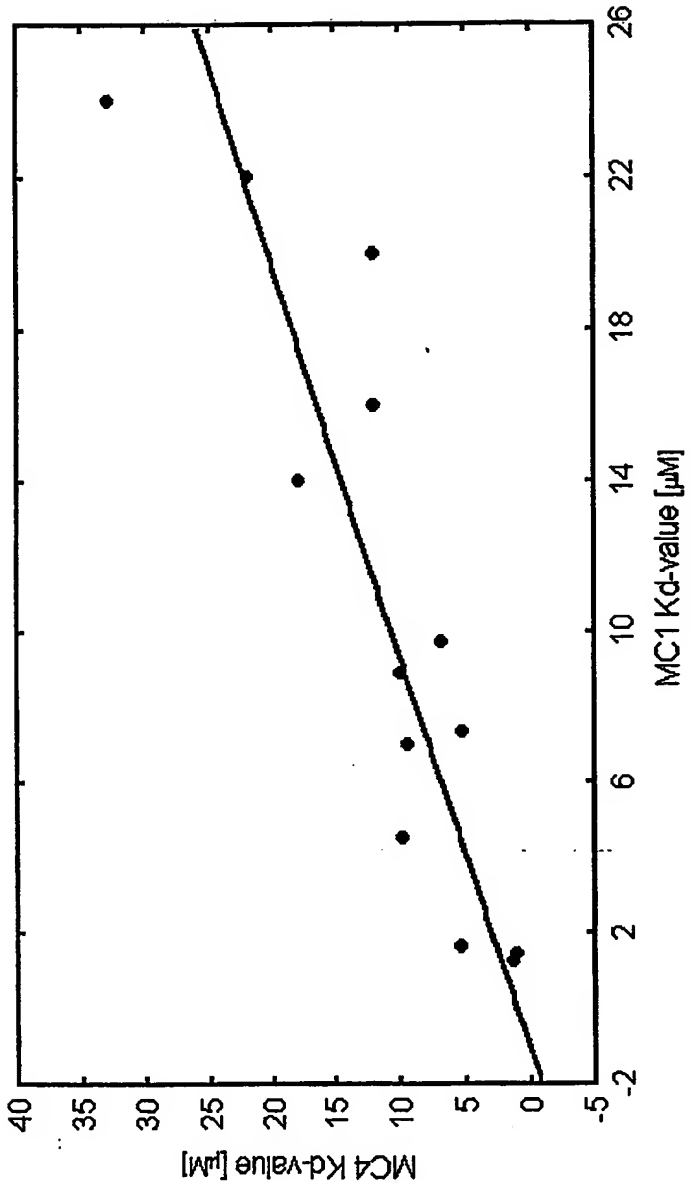

MC4 Kd-value [µM]
40
35
30
25
20
15
10
5
0
-5
MC1 Kd-value [µM]
26
22
18
14
10
6
2
-2

Fig. 8

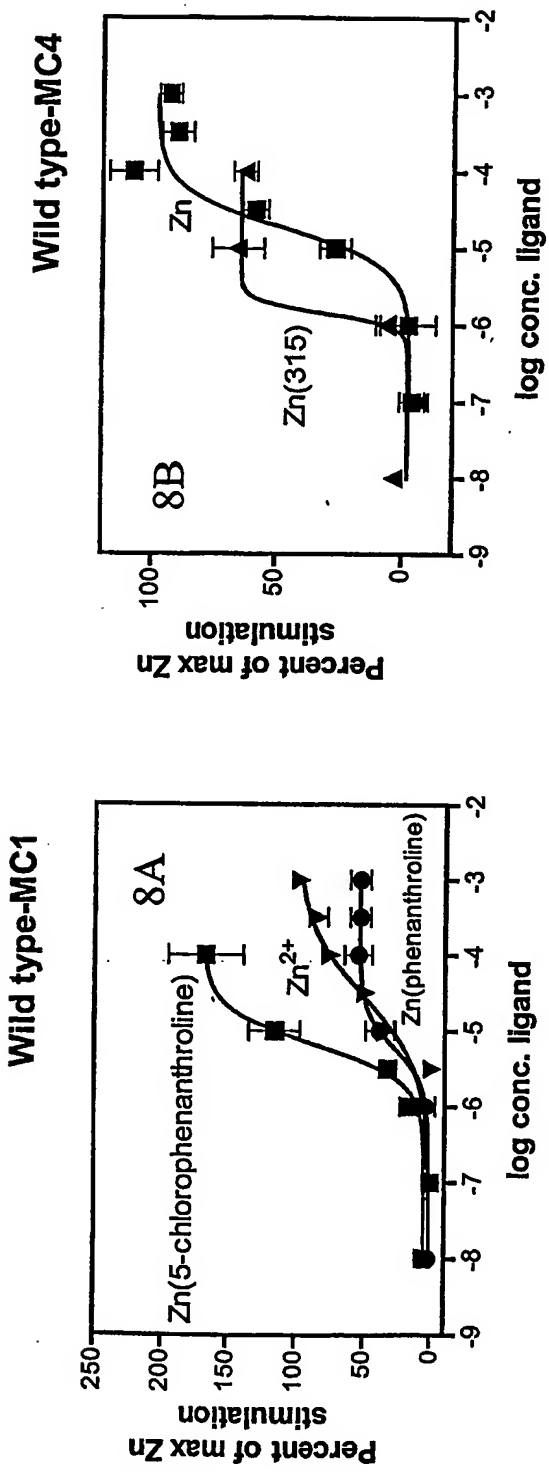

Wild type-MC4
8B
Zn
Zn(315)
Percent of max Zn stimulation
log conc. ligand
Wild type-MC1
8A
Zn(5-chlorophenanthroline)
Zn2+
Zn(phenanthroline)
Percent of max Zn stimulation
log conc. ligand

Fig. 9

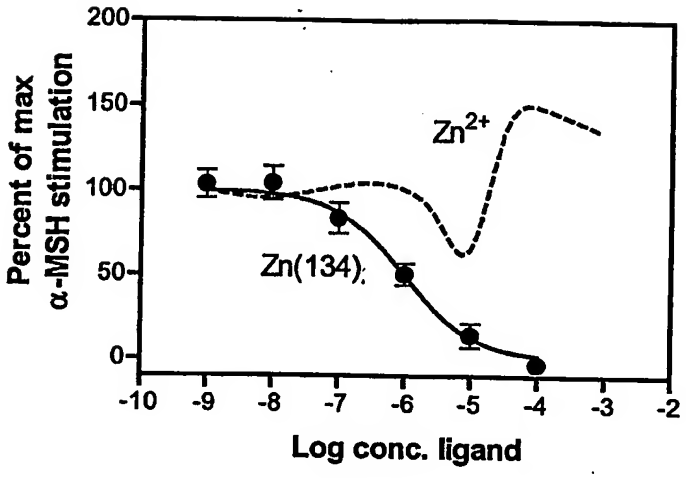

200
150
100
50
0
Percent of max
α-MSH stimulation
Zn2+
Zn(134)
-10
-9
-8
-7
-6
-5
-4
-3
-2
Log conc. ligand

Fig. 10

INTERNATIONAL SEARCH REPORT

Internat pplication No
PCT/DK 02/00902

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/33 A61K31/4427 A61P3/04 A61P3/06 A61P3/10
A61P9/00 A61P15/10 A61P19/02 A61P19/06 A61P37/02
//A61K31/045,A61K31/095,A61K31/66

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07D C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 13112 A (CAI HUI ZHI ;SHARMA SHUBH D (US); SHI YI QUN (US); YANG WEI (US);) 22 February 2001 (2001-02-22) page 1-28; claims 1-17; figures 1-7	1-28
Y	---	1-28
Y	WO 01 50127 A (SCHWARTZ THUE W ;7TM PHARMA (DK); ELLING CHRISTIAN E (DK); GERLACH) 12 July 2001 (2001-07-12) page 1, line 5-18 page 2, line 15-17 page 5, line 18-22 page 6, line 17 -page 11, line 32 especially page 15, lines 32 and 35-36; pages 30-32 and figures III.3C-III.4C and V-VI.	1-28
X	--- -/--	27,28

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 April 2003	12. 05. 2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	PER RENSTRÖM / ELY

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01 60349 A (NOVACTYL INC) 23 August 2001 (2001-08-23) page 1-10; claims 1,3-5,7	1,5,6, 12-18, 23-26,28
X	US 5 401 746 A (FEY PETER ET AL) 28 March 1995 (1995-03-28) abstract, examples 183-184, col. 116 (chelators for the treatment of arteriosclerosis, a cardiovascular disease)	1,6, 12-14, 23-26,28
X	US 6 284 795 B1 (SEXTON KAREN ELAINE ET AL) 4 September 2001 (2001-09-04) abstract, example 1, col. 8 (a chelator for the treatment of atherosclerosis, a cardiovascular disease)	1,6, 12-14, 23-26,28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 02/00902

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-2,4-16,18-25
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 1-28
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1,6,12-14,23-26, and 28 and the whole of claims 3 and 4

the inventions according to parts of claims 1, 6, 12-14, 23-26 and 28 and the whole of claims 3 and 4, relating to the problem of treating overweight, obesity, hypercholesterolemia, hypertriglyceridemia, or reducing the fat tissue mass/ lean mass body mass ratio;

2. Claims: 1,6,12-14,23-26 and 28 and the whole of claim 2

the inventions according to parts of claims 1, 6, 12-14, 23-26 and 28 and the whole of claim 2, relating to the problem of treating diabetes type 2;

3. Claims: 1,6,12-14,23-26 and 28

the inventions according to parts of claims 1, 6, 12-14, 23-26 and 28, relating to the problem of treating cardiovascular diseases and hypertension;

4. Claims: 1,6,12-14,18,23-26 and 28

the inventions according to parts of claims 1, 6, 12-14, 18, 23-26 and 28 and the whole of claims 5, 15-16 relating to the problem of treating arthritic diseases, conditions involving the immune system and chronic and acute inflammation;

5. Claims: 7-8 and parts of claims 12-14,23-26 and 28

the inventions according to the whole of claims 7-8 and parts of claims 12-14, 23-26 and 28, relating to the problem of treating anorexia and/or other appetite disorders;

6. Claims: 9-11 and parts of claims 12-14,23-26 and 28

the inventions according to the whole of claims 9-11 and parts of claims 12-14, 23-26 and 28, relating to the problems of treating sexual dysfunction / erectile dysfunction;

7. Claims: 19-20 and parts of claims 12-14,23-25 and 28

the inventions according to the whole of claims 19-20 and

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

parts of claims 12-14, 23-25 and 28, relating to the problem of treating steroidal disorders;

8. Claims: 2122 and parts of claims 12-14,23-25 and 28

the inventions according to the whole of claims 21-22 and parts of claims 12-14, 23-25 and 28, relating to the problem of treating perspiration disorders;

9. Claims: 17 and parts of claims 12-14,18,23-25 and 28

the inventions according to the whole of claim 17 and parts of claims 12-14, 18, 23-25 and 28, relating to the problem of obtaining a suitable tan of the skin;

10. Claim : 27

the inventions according to the whole of claim 27, relating to the problem of using a chelate and/or chelator as a lead compound in a drug discovery process for identifying ligands that interact with an MC receptor.

The common general inventive concept (see PCT Rules 13.1 and 13.2) linking together the problem-derived groups of inventions listed above is the solution in the form of the use of a chelate and/or a chelator which is capable of interacting with the metal-ion binding site in an MC receptor. Since the search revealed that this technical feature is not novel, the invention is considered to lack a single general inventive concept according to the requirements of unity of invention in PCT Rules 13.1 and 13.2.

Although the requirements for unity of invention are not fulfilled, the International Searching Authority has chosen not to invite payment of additional fees. However, independently of the non-unity of invention, the search has been severely restricted due to the lack of clarity and conciseness of the claims - see Box 1 for further information.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 1-2,4-16,18-25

Claims 1-2,4-16,18-25 relate to methods of treatment of the human or animal body by surgery or by therapy/diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

Continuation of Box I.2

Claims Nos.: 1-28

Present claims 1-25 and 27-28 relate to compounds defined by reference to a desirable property, namely that the compounds should be capable of interacting with the metal-ion binding site in an MC receptor. An attempt is made to define the compounds by reference to a result to be achieved. This is not an accepted way of defining compounds, since there may well be compounds having the said property without this being reported, thereby making it impossible to perform a complete search.

Present claims 1-28 relate to methods using an extremely large number of possible compounds, due to the extreme broadness in scope inherent in the terms "chelate" and "chelator", covering essentially all organic compounds containing two or more metal- or metal-ion-binding heteroatoms (N, O, S, Se, P etc.) in accessible positions, of which compounds only a small proportion is probably being reported as "chelates" or "chelators". The general formula I in claims 25 and 28 also relate to an extremely large number of possible compounds. The broadness of the claims makes a meaningful search over the whole of the claimed scope impossible, and support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is furthermore to be found only for methods using a very small proportion of the compounds.

Furthermore, the claims containing the expressions "complications thereto" (claims 1, 6, 12-14, 23-26 and 28), "conditions involving the immune system" (claims 5-6, 12-15, 23-26 and 28), "sexual dysfunction" (claims 9, 11-14, 23-26 and 28), "steroidal disorders" (claims 19-20, 23-25) and "perspiration disorders" (21-25) are not considered to be clear and concise according to Article 6 PCT since the said terms do not unambiguously define specific pathological conditions, and the claims have accordingly not been searched completely.

The search has been restricted to (1) methods using compounds of the bipyridine type in Examples 1-31 on pages 38-76 in the application as well as the corresponding 2-(pyrrol-2-yl)-pyridines and 2-(thien-2-yl)-pyridines, and (2) general keywords such as chelate, metal/metal-ion binding, metal/metal-ion site, bidentate, MC receptor, melanocortin, POMC, adrenocorticotropic, ACTH, melanocyte stimulating and

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

drug discovery / screening / identification / optimization; and for claims 1, 5-6, 9, 11-15, 19-26 and 28 further restricted by the lack of clarity and conciseness of the above mentioned terms relating to pathological conditions.

Finally, attention is drawn to the fact that the present claims 6, 12-14, 18 and 23-25 refer to both cosmetic and therapeutic methods.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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Information on patent family members

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